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EXPERIMENTAL DIVISION OF THE SHOOT APEX OF *LUPINUS ALBUS* L.[1]

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The differentiation of the primary shoot appears to be the result of development of tissues produced by the shoot apex. The importance of this permanently embryonic region (the dome of tissue above the youngest foliar primordia) was long ago emphasized by Goebel (1928) and by Bower (1947). The shoot apex consists of young cells that histologically appear to resemble each other closely. On proceeding downward from this embryonic region the primary shoot shows more and more differences between the kinds of cells present until the mature state is attained. In the primary shoot, the tissues left behind by the growth of the terminal bud are mostly living. It is the suggestion of some investigators (Bower, 1930), (Dixon, 1936), that these subjacent, living tissues of the primary shoot exert a controlling effect in an upward direction upon the form-building activities of the shoot apex. Anatomical data relating to the course of development of procambium have generally been taken to support this conception.

The alternate hypothesis to account for the differentiation of tissues below the shoot apex is that this meristem is not only producing the cells but is also determining the manner in which they mature. This hypothesis has been supported by some anatomical investigations which show basipetal determination of procambium from foliar primordia on the shoot apex (Gráf, 1938; Helm, 1931; Herrig, 1915; Hsü, 1944). Evidence that supports this hypothesis was given by Ball (1949) for *Lupinus albus*. Experimental techniques applied to the shoot apex have also given support to this hypothesis (Snow and Snow, 1947; Ball, 1947, 1948, 1950, 1952). Wardlaw's experiments (1944, 1946a, 1946b, 1947, 1948, 1949) on the fern shoot apex, which

[1] Communicated to the Seventh International Botanical Congress, Stockholm, 1950. Contribution from the North Carolina Agricultural Experiment Station. Published with the approval of the Director as Paper No. 438 of the Journal Series.

differs in some respects from the angiospermous shoot apex, support the hypothesis of determination of subjacent tissues by the shoot apex.

Previous work by the writer (1946) has shown that an entire shoot apex plus the three youngest foliar primordia could grow *in vitro* into an entire plant. Pilkington (1929) demonstrated that halving this shoot apex is followed by regeneration of each half into a complete shoot apex. It was subsequently shown by Ball (1948) that this shoot apex, if divided into quarters, would show regeneration in each quarter into a normal apex that produced a normal shoot. In the same communication (also Ball, 1952) the experiment of isolation of the central portion of the shoot by four longitudinal cuts was described; this portion gave rise to an apex that produced a shoot. Removal or isolation by diagonal cuts of the central portion of this apex (Ball, 1950) was followed by regeneration of the lateral portions of the original meristem into shoot apices that grew into normal shoots. It has thus been demonstrated that this shoot apex has the capacity for producing more than one shoot apex and primary shoot and that these shoots are produced by the activities of the new regenerated apices and are not determined morphogenetically by the previously existing tissues of the original shoot. It is perhaps natural to conceive that the mature, primary shoot might determine the terminal shoot apex to produce a continuation of the same pattern in the new tissues of a single shoot. But when a shoot apex is divided and each subdivision of the original meristem regenerated into a new, normal shoot apex that produces a normal shoot on top of the original one, it lends strong support to the view that the distal meristem, given adequate nutrition from below, is a self-determining morphogenetic region.

In view of the multicellular, layered construction of the dicotyledonous shoot apex, a question of some relevance is: what is the minimum size of a portion of this apex that will still regenerate into an apex that will produce a shoot? In addition to the importance of finding the minimum volume of meristem and the minimum number of meristematic cells that will regenerate an apex, there was the possibility that the new meristems might during their early growth be significantly smaller than the normal. Shoots produced from such small apices might be very instructive in the question of the morphogenesis of tissues in the primary shoot: do the subjacent tissues determine the form of future tissues, or does the shoot apex function independently in this respect?

Earlier experiments in dividing this shoot apex had shown that upon regeneration of the fraction into a functional shoot apex, the first procambium was a complete ring in the case of quartering (Ball, 1948) or a ring only under the part of the new apex above the position of the wound in the case of removal of the central portion of the original apex (Ball, 1950). These rings of procambium were transitory and the mature shoots produced by the regenerated apices displayed the normal separate bundles of vascular tissues. The possibility was entertained in planning the present research that, if shoot apices could be regenerated from the minimum number of meristematic cells of an original apex, the first-formed vascular system might mature as a tube of vascular tissues or even as a solid rod of vascular tissues. These types of vascular tissues would constitute distinctive patterns for this plant, and would serve as markers delimiting the first differentiative activities of the regenerated apices.

Materials and Methods.—Potted plants approximately thirty days old were used. After moving aside the foliar primordia that covered the apices, the latter were cut into eight or six equal parts by a microscalpel (see Ball, 1946, for method of making this tool) by cuts approximately 100μ deep (Fig. 1). All operations were done under a binocular dissecting microscope with a magnification of 140×. Lighting was from a Spencer Universal Lamp with a heat-absorbing glass which gave a spot that became no warmer than 30° C. After the operations the leaves were bent back into their original positions and tied with a soft cotton cord that was left for forty-eight hours. At various numbers of days after the operations during the next two months the buds of these plants were excised, killed, dehydrated, infiltrated with paraffin, sectioned and stained.

Experimental Results.—Dividing of the shoot apex into eight parts was not usually followed by regeneration of any of the subdivisions into apices. The usual growth of such operated plants was the precocious sprouting of one or more uppermost axillary buds. This part of the experimental procedure was therefore abandoned, with the tentative conclusion that one eighth of an apex of this plant is too small a volume of meristematic tissue to regenerate an apex.

Dividing of the shoot apex into six parts gave volumes of meristem large enough to regenerate new apices. One hundred and eight plants were analyzed by making serial sections. In most cases, from one to five of the divisions of the original meristem regenerated into func-

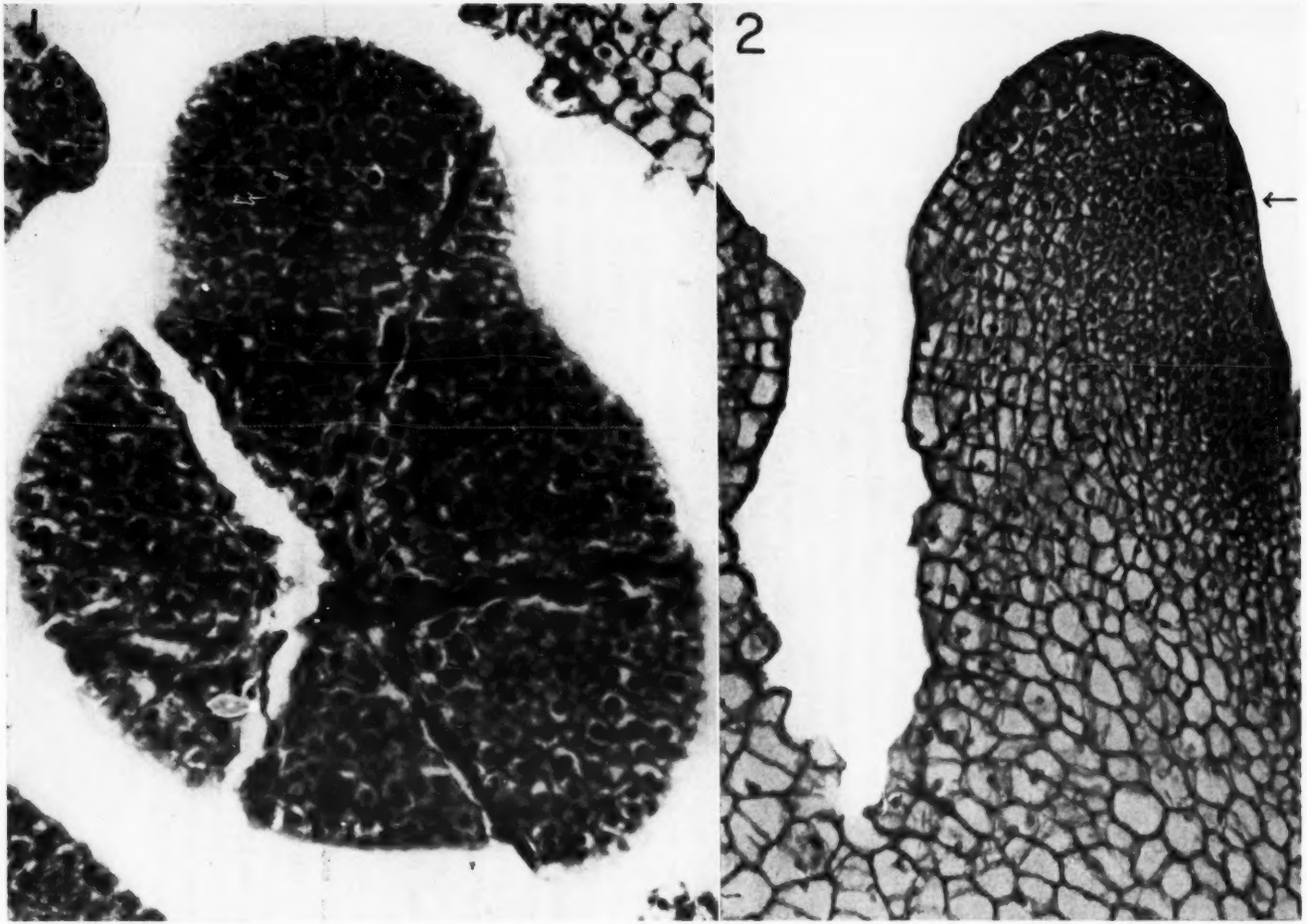


FIG. 1.—Transverse section of a shoot apex that was killed immediately after its division into six parts. The portions are not all equal in size. 510×. FIG. 2.—Longitudinal section of a sixth of an apex two days after the operation. The segment had elongated considerably above its original level, so that the group of new initial cells (beyond arrow, in second tunica layer and in corpus) was separated from the subjacent, pre-operation procambium. 282×.

tional apices (Fig. 3). Of the total theoretically possible six hundred and forty-eight regenerated apices from this group of experimental plants, either new shoot apices or new shoots were found in two hundred and sixty-eight cases. It is not apparent why all six of the divisions of one original apex did not regenerate; perhaps there was an inhibition of the growth of some of the portions by those that had first begun to grow. Also, it is probable that some of the portions of the original apex were below the minimum size for regeneration because of inequalities of the operation (cf. Fig. 1).

Soon after the operation was performed the portions of the original apex elongated considerably (Fig. 2). The regeneration of the portions into new apices began by definition of a group of new initial cells by divisions in the second tunica layer and in the corpus (Fig. 2). Because of the early elongation, these new groups of initials were a considerable distance above the subjacent original procambium. This formation of new initials from the flank or lateral cells of the original apex was the same as that described in the regeneration of new apices after removal or isolation of the central sector of the apex (Ball, 1950). Such regeneration is evidence that the lateral cells of this apex, although they normally form only foliar primordia, have the potentiality of producing a shoot apex. There was thus no irreversible development for these cells; they could, depending upon the position in which they found themselves, produce either a foliar primordium or a shoot apex.

The portions that were obviously above the minimum size regenerated to a size approaching that of normal apices and had a transitory ring of procambium beneath them (Fig. 4, a and b). There was an early increase in diameter of these apices. Two hundred and forty-three of the two hundred and sixty-eight regenerated apices were of this type. The average diameter of ten of these apices was 127μ. Such apices produced normal-sized shoots (Fig. 12, a, b, c) which were found on sectioning to have separate bundles of vascular tissue in a ring surrounding a wide pith. The smaller portions of the original apex regenerated small shoot apices (Fig. 4, c and d). Twenty-five of the two hundred and sixty-eight regenerated apices were of this type. In these small apices there was no early increase in diameter, but the size remained that of the original capital—approximately one-sixth of the original meristem. The average diameter of six of these small apices was 70μ. Such apices produced shoots that were of

size. 510×.

FIG. 2.—Longitudinal section of a sixth of an apex two days after the operation. The segment had elongated considerably above its original level, so that the group of new initial cells (beyond arrow, in second tunica layer and in corpus) was separated from the subjacent, pre-operation procambium. 282×.

smaller than normal diameter (Fig. 12, d). They will be referred to as thin shoots. In every bud analyzed a few days after the operation, there were one or more portions of the original apex that had not regenerated (Fig. 4, e and f) but in which the cells had enlarged and vacuolated. Such portions were presumably below the minimum number of meristematic cells for regeneration into a functional apex. Although the above sequence of events in regeneration was often found in the analyzed buds, frequently the first-growing portions of the original apex regenerated into small shoot apices. The immediate subjacent differentiation of these small shoot apices was a suspended rod of small, densely-staining cells, which are interpreted by the writer as procambium. In a transectional series of a bud four days after the operation, the rod of procambium was first detectable 70μ below the tip of the regenerated apex (Fig. 5), was clearly distinguishable 100μ below the tip (Fig. 6) and was not present 160μ below the tip (Fig. 7). The fact that this strand of procambium was suspended, i.e., not attached basally to subjacent, older procambium or vascular tissues, is evidence that it had been produced by the shoot apex, and that the direction of differentiation of this procambium was basipetal.

The early growth of the small shoot apex, before the formation of leaves, produced the rod of procambium.

Continued growth of the small shoot apex brought about basal connection of the rod of procambium to subjacent procambium. In a series of transections taken of a bud eight days after the operation, Fig. 8 shows the small apex and a foliar primordium, Fig. 9 (20μ down) shows the base of the apex, Fig. 10 (80μ down) the slender, subjacent shoot with the central rod of densely-staining procambium, and Fig. 11 (280μ down) shows the connection of this rod of procambium (at a) with the subjacent procambium that was present at the time of the operation. There were five regenerated shoot apices from this operation; they were either small and produced rods of procambium that connected with the basal stem as at a and b, or they regenerated into normal apices that produced a subjacent ring of procambium as at c.

The formation of leaves on the small shoot apices was very instructive, because the leaves were formed laterally on the meristem while the subjacent procambium was a centrally located rod (cf. Figs. 8, 9, 10). The form and position of previously produced procambium obviously had no influence upon the origin of foliar primordia as

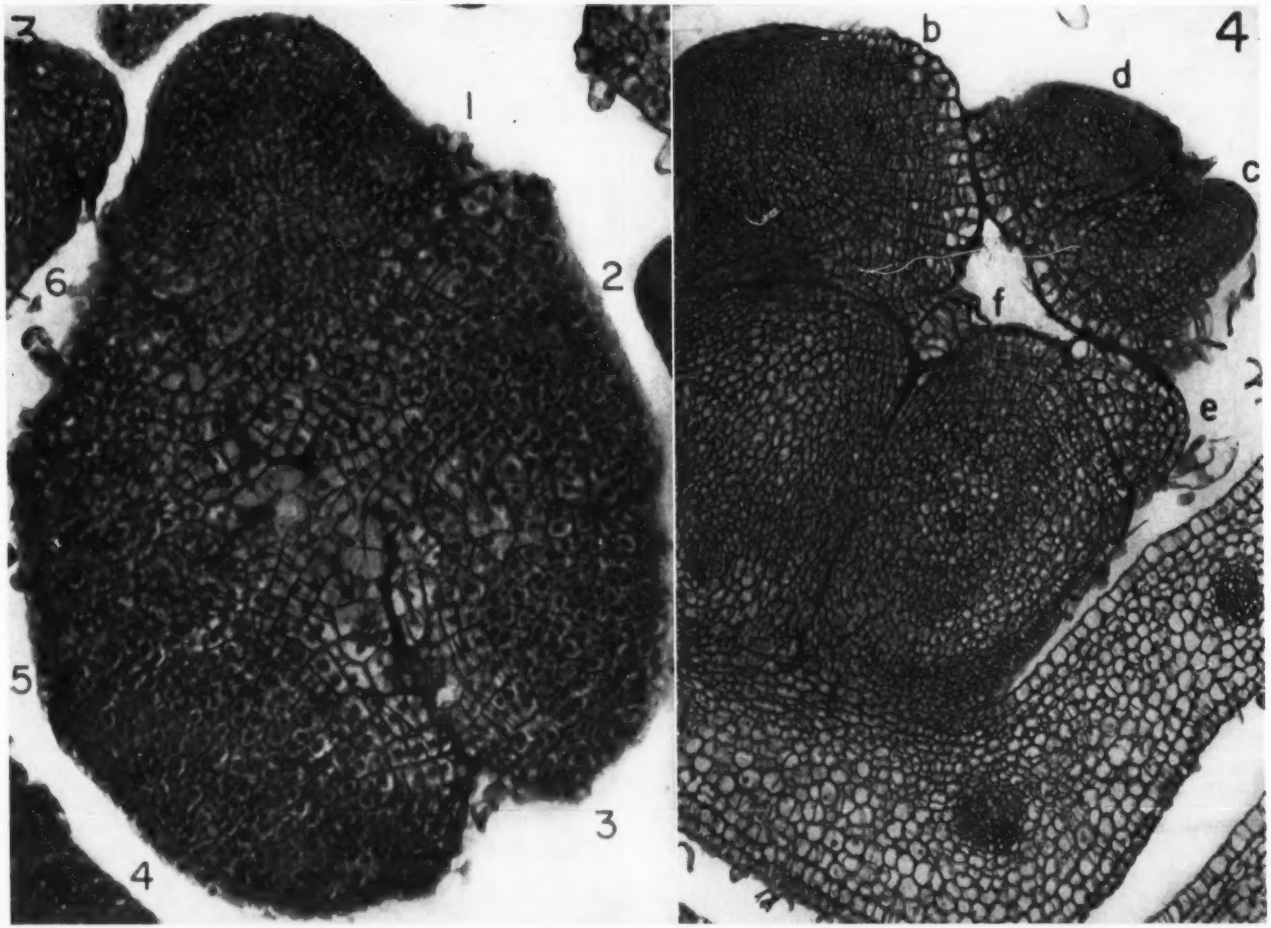


FIG. 3.—Transverse section of a bud three days after the operation. To the outside of sector 1 is the foliar primordium that was present on the original apex at the time of the operation. Sectors 2, 3, 4, and 6 appear to have shown growth by cell division. 282×. FIG. 4.—Transverse section of a bud seven days after the operation. Two of the apices (a and b) had become large and approximately normal and had produced rings of subjacent procambium. Two of the apices (c and d) were small, and the last two of the original six divisions (e and f) had failed to regenerate and their cells had enlarged and vacuolated. 250×.

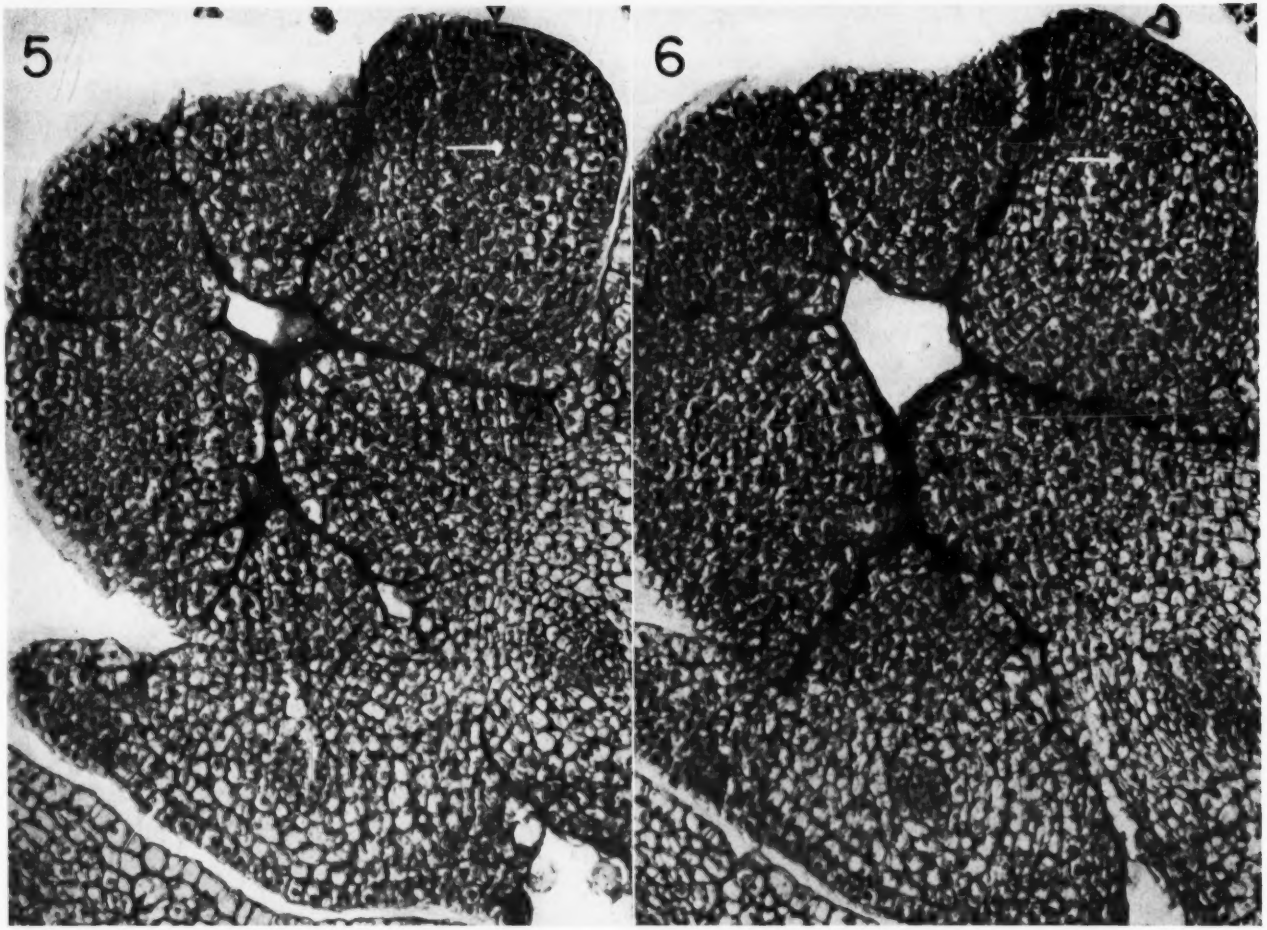
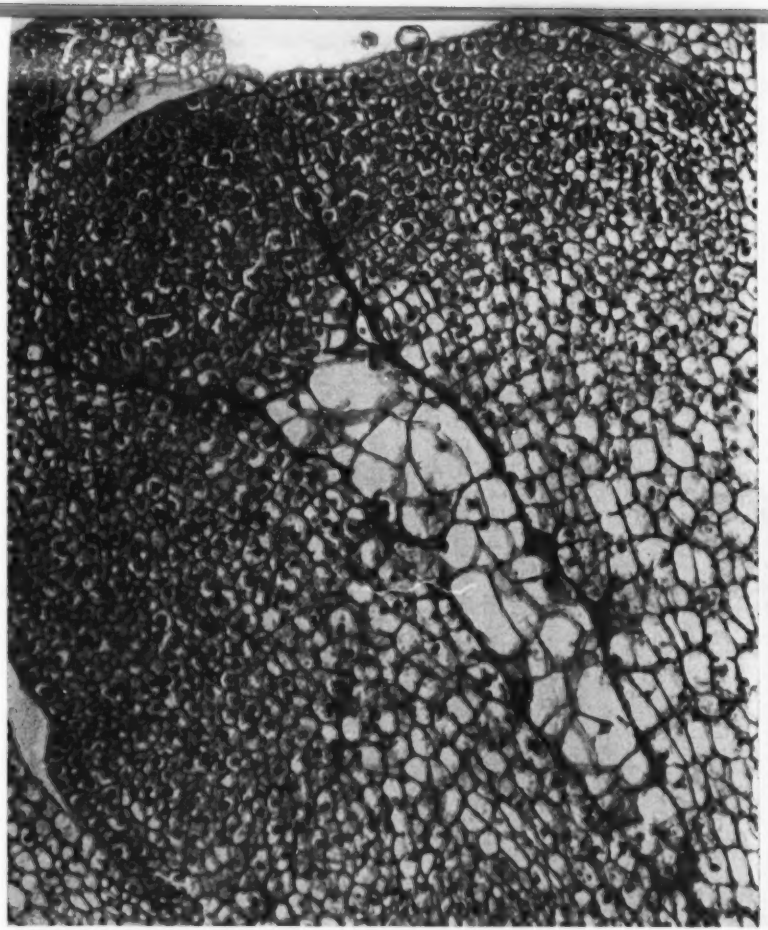


FIG. 5.—Transection of a bud four days after the operation. The upper right sector is shown 70μ below the terminal meristem, which was a small apex. The arrow indicates the beginning of the rod of subjacent procambium. 282×. FIG. 6.—Transverse section of the same bud 30μ below the last figure. The upper right sector is 100μ below the small apex that regenerated from one-sixth of the original shoot apex. The arrow indicates the rod of subjacent procambium that was differentiated below this apex. 282×.



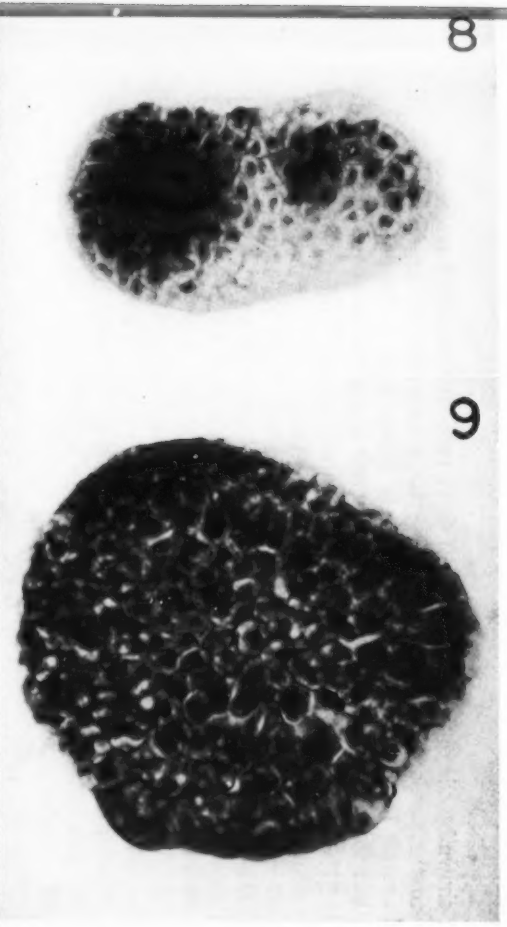


FIG. 7.—Portion of transverse section of the same bud shown in Figs. 5 and 6, but 160μ below the small apex. The upper right part of the figure corresponds to the same sector of Figs. 5 and 6, but there is no procambium. The rod of procambium in this young shoot was therefore a suspended strand, since it did not connect at this early stage with subjacent provascular tissues. 282×. FIG. 8.—Transverse section of a small shoot apex (63μ in diameter) that had regenerated from one-sixth of an original shoot apex eight days after the operation. To the right is shown the first foliar primordium produced by this apex. 545×. FIG. 9.—Transverse section of the base of the small shoot apex shown in Fig. 8, 20μ below the tip. No procambium is evident at this level. 545×.

shown by the different positions of these two structures with reference to the shoot apex and also because the provascular tissue first appeared 70-100μ below the level of the primordium. The process of leaf formation appeared to be determined entirely by the shoot apex.

The maturation of the rod of procambium beneath the small apex occurred into either a tube of vascular tissues (Fig. 15) or a solid rod of vascular tissues (Fig. 13) in the shoots that have been analyzed. The shoots of smallest diameter contained the rods, while those of larger diameter contained the tubes. There was thus a size relationship between the diameter of the stem and the type of stele contained. This size relationship may have been determined by the size of the regenerated shoot apex that produced the shoot. This, however, is difficult to establish, since it was impossible to determine by observing the procambium whether it would have matured into a tube or a rod of vascular tissues.

The shoots produced by these small shoot apices were analyzed at twenty-one and forty-nine days after the operations. The forty-nine day-old shoot (Fig. 12, d) that will be used for illustrations had a tube of vascular tissue with a conspicuous pith at its base immediately above the level of its attachment to the original shoot (Fig. 14). Farther up (4.3 mm.) the shoot, the stele had a smaller diameter and the pith was much narrower (Fig. 15). The veins of the first two leaves produced on this shoot appeared to be independent of the stele that first had been formed by the shoot apex. Fig. 16 shows the lowest extension (7.5 mm. above the base and 2.2 mm. below the shoot apex) of the trace of the second leaf and its blind ending. Fig. 17 (8.1 mm. above base) shows the trace of the second leaf and the stele of the stem. At 8.4 mm. (Fig. 18) above the base was found the union of the traces of the first two leaves. This union was of the nature of a lateral fusion of already-established vascular strands, and constituted the upper limit of the stele of the thin shoot. Above that level, with the production of additional foliar primordia by the shoot apex, there was formed an eustele. Wardlaw (1944, 1946a) established that certain fern apices with primordia constantly removed showed a change from dictyostele to solenostele in the subjacent shoots. When primordia were permitted to remain on the apices and to develop into leaves, the vascular tissue beneath the terminal meristem again became a normal dictyostele. Fig. 19 (8.6 mm. above base) shows the beginning of the ring of bundles. The further development of the ring of

bundles is seen in Fig. 20 (8.8 mm. above base). Fig. 21 (8.9 mm. above base) shows the ring of bundles symmetrically and the traces of the first two leaves. The last-formed procambium of this shoot is seen to be normal in that there are bundles in a ring surrounding a wide pith (Figs. 22 and 23, 9.2 and 9.5 mm. respectively, above base). The last of this series, Fig. 24 (9.7 mm. above base) shows the shoot apex and the youngest foliar primordia. The shoot apex was 146μ wide. The latter is closely comparable to the range of diameter, 150-200μ, found in the normal shoot apex of this plant (Ball, 1949). A great difference is noted between the diameter of this experimental shoot apex and the average of the small shoot apices (70μ). The shoot apex that produced the shoot shown in Figs. 14-24 probably started its growth as a small meristem comparable to Fig. 8, since it must have produced a rod of procambium. The shoot apex of this forty-nine day-old shoot must have undergone considerable increase in diameter during its growth and change from the production of the rod of procambium to the eustele. It would appear that this shoot apex had during this interval become essentially normal.

Discussion

The dividing of the shoot apex into six parts gave fractions that were each of about 60° of arc. Since there were, in every bud that was analyzed fractions too small to regenerate, some that regenerated into normal apices, and some that regenerated into small apices, it can be concluded that such segmentation represented approximately the minimum volume of meristem for regeneration of an apex.[1] These results give an experimental basis for the observation that the shoot apex of an angiosperm apparently grows by the activity of numerous cells, for reduction of the cell number below the minimum resulted in failure of the segment to show the characteristics of an apical meristem. These results further demonstrated the unimportance of any individual cell in the apex, and showed that there must be a minimum number of cells working together to constitute an apex. These ideas have a parallel in the early controversy between the adherents of the apical cell theory and those of the histogen theory (cf. Foster,

[1]Sussex (*Nature* **170**: 755) found that 1/6 of the shoot apex of potato was the approximate minimum for regeneration of an apex when the rest of the original apex was left intact. If the latter were removed, smaller panels (twelve surface cells, equivalent to ca. 1/20 of original apex) would regenerate apices.

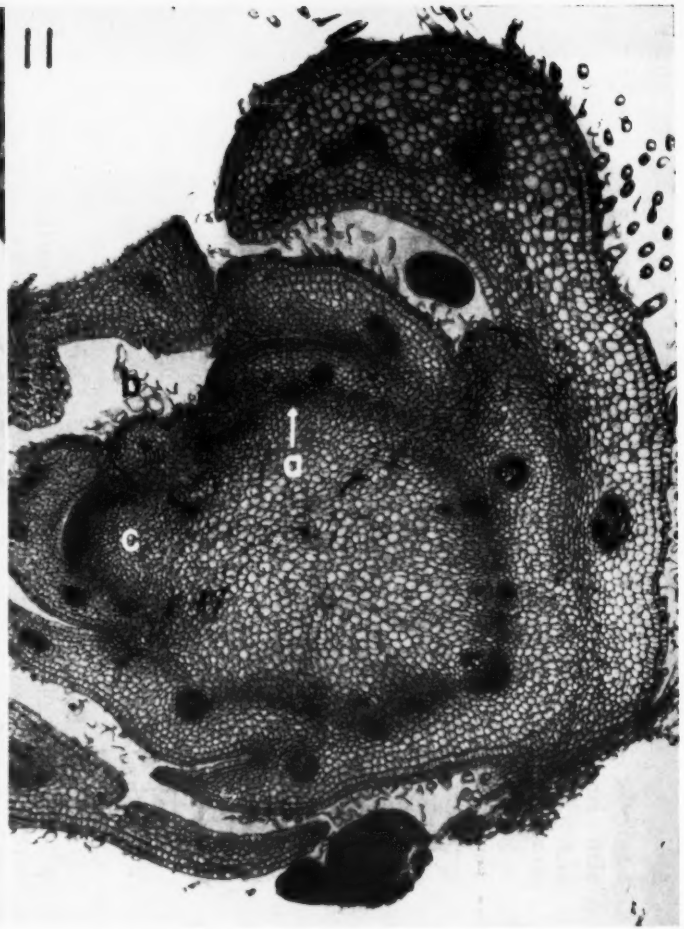


FIG. 10.—Transverse section of the thin shoot that had been produced by the small apex of Fig. 8, at 80μ below the terminal meristem. The procambium is evident as a rod of small, densely-staining cells in the center of the shoot. 545×. FIG. 11.—Transverse section of the shoot 280μ below the level of Fig. 8. At (a) is shown the procambium from the shoot of Fig. 10 connecting with the preoperation procambium. There were two thin shoots produced from the segments of the original apex (a and b). Three other shoots that had grown from the segments of the original apex were normal (the base of one is seen at c). 65×.

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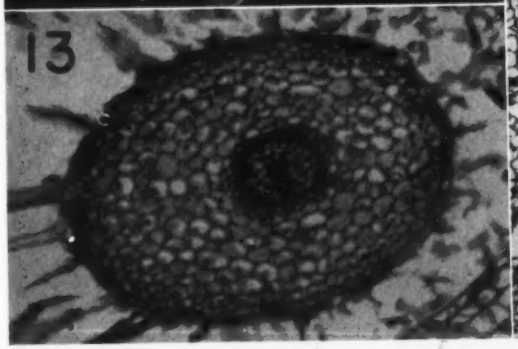

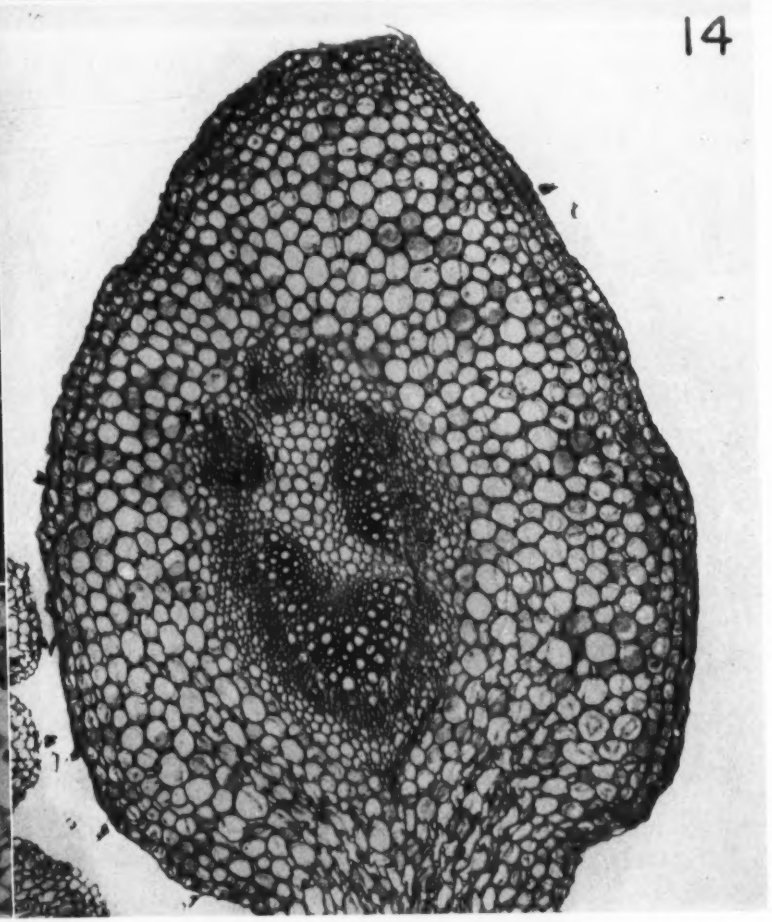

FIG. 12.—View of four shoots that had been produced from sixths of an original apex 48 days after the operation. The shoots a, b and c are normal-sized, but d is a thin shoot. The serial transverse sectional series in Figs. 14-24 was made from this shoot. 1/6×. FIG. 13.—Transverse section of a thin shoot killed 21 days after the operation. The rod of procambium had matured as a rod of vascular tissue. 130×. FIG. 14.—Transverse section of the base of the thin shoot shown in Fig. 12 d. The stele is a tube of vascular tissues with a wide pith. 65×.

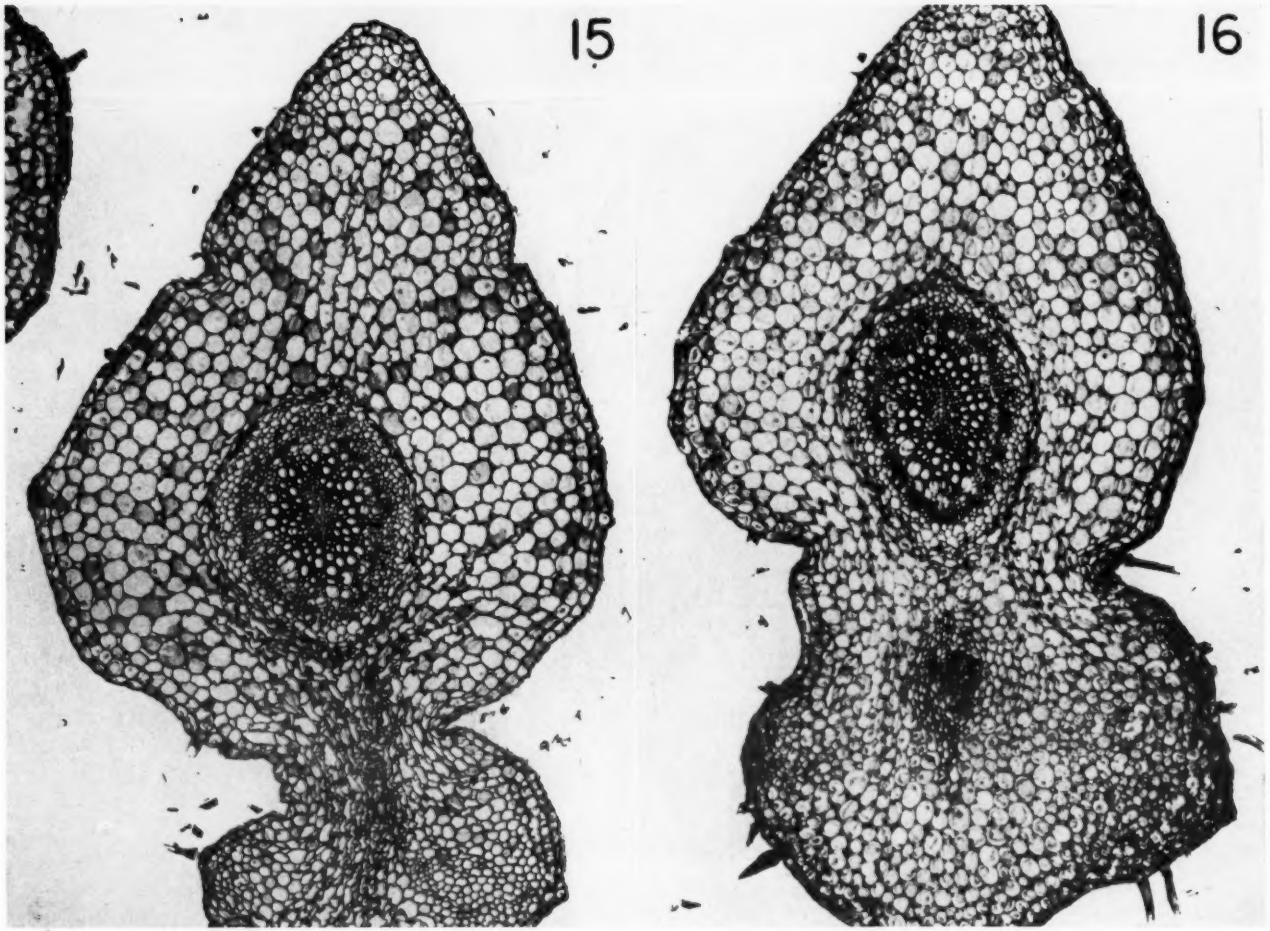

FIG. 15.—Transverse section of the thin shoot 4.3 mm. above the base. The stele is much narrower, the pith has a smaller diameter and is composed of very small parenchymatous cells. 65×. FIG. 16.—Transverse section of the thin shoot 7.5 mm. above base. The stele is approximately the same as in the preceding figure. Lower part of the figure shows the leaf base and the lowest extension and blind ending of the trace of the second leaf. 65×.

of very small parenchymatous cells. 65×. FIG. 16.—Transverse section of the thin shoot 7.5 mm. above base. The stele is approximately the same as in the preceding figure. Lower part of the figure shows the leaf base and the lowest extension and blind ending of the trace of the second leaf. 65×.

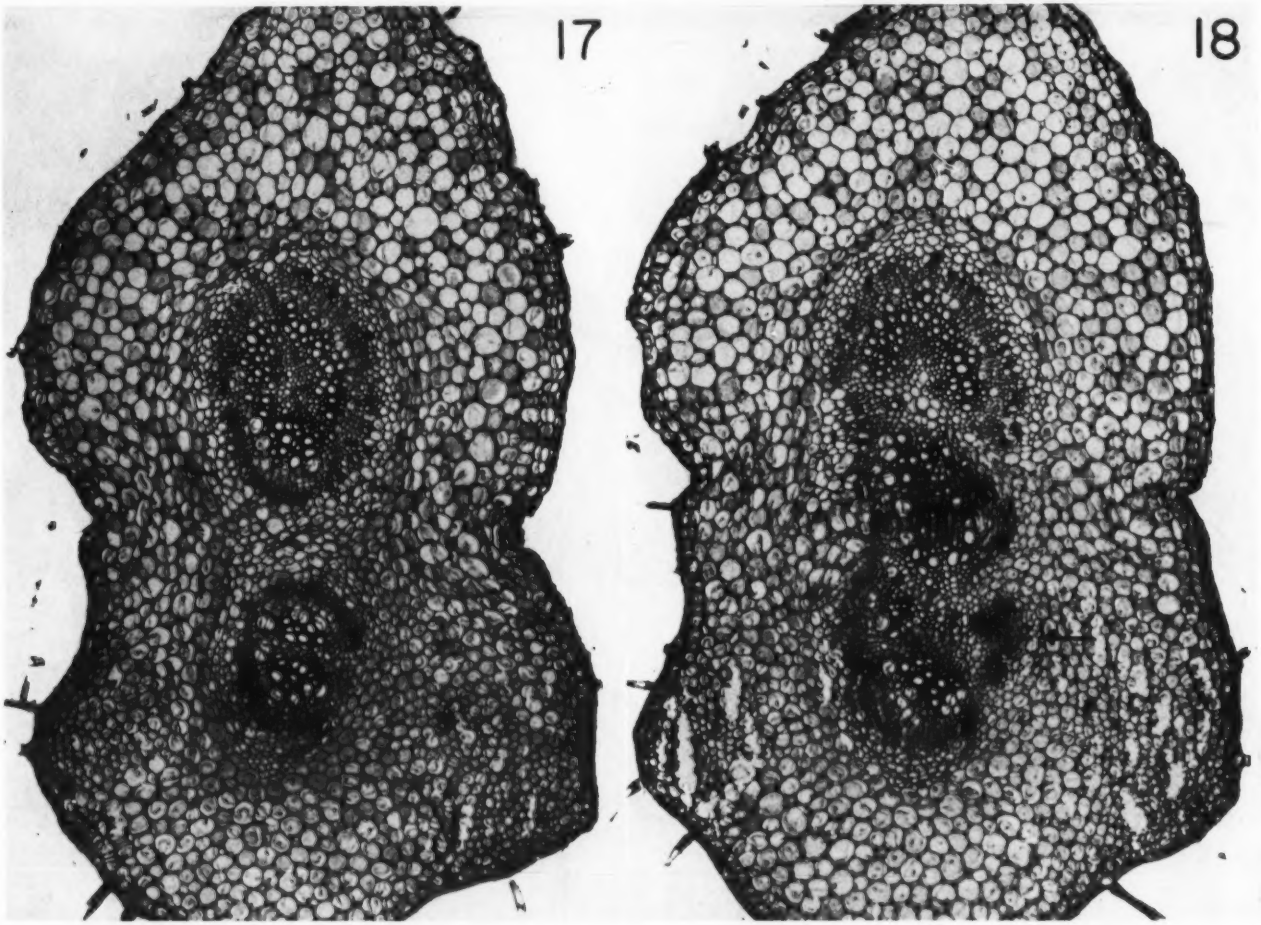

FIG. 17.—Transverse section of the thin shoot 8.1 mm. above base, showing the stele (above) and the trace of the second leaf (below). 65×.
FIG. 18.—Transverse section of the thin shoot 8.4 mm. above base. The trace of the first leaf (above) and that of the second leaf (below) are fused by a central connection. Arrow points to the beginning of the ring of bundles that will become evident in sections above this level. This level was the upper limit of the stele of the stem. 65×.

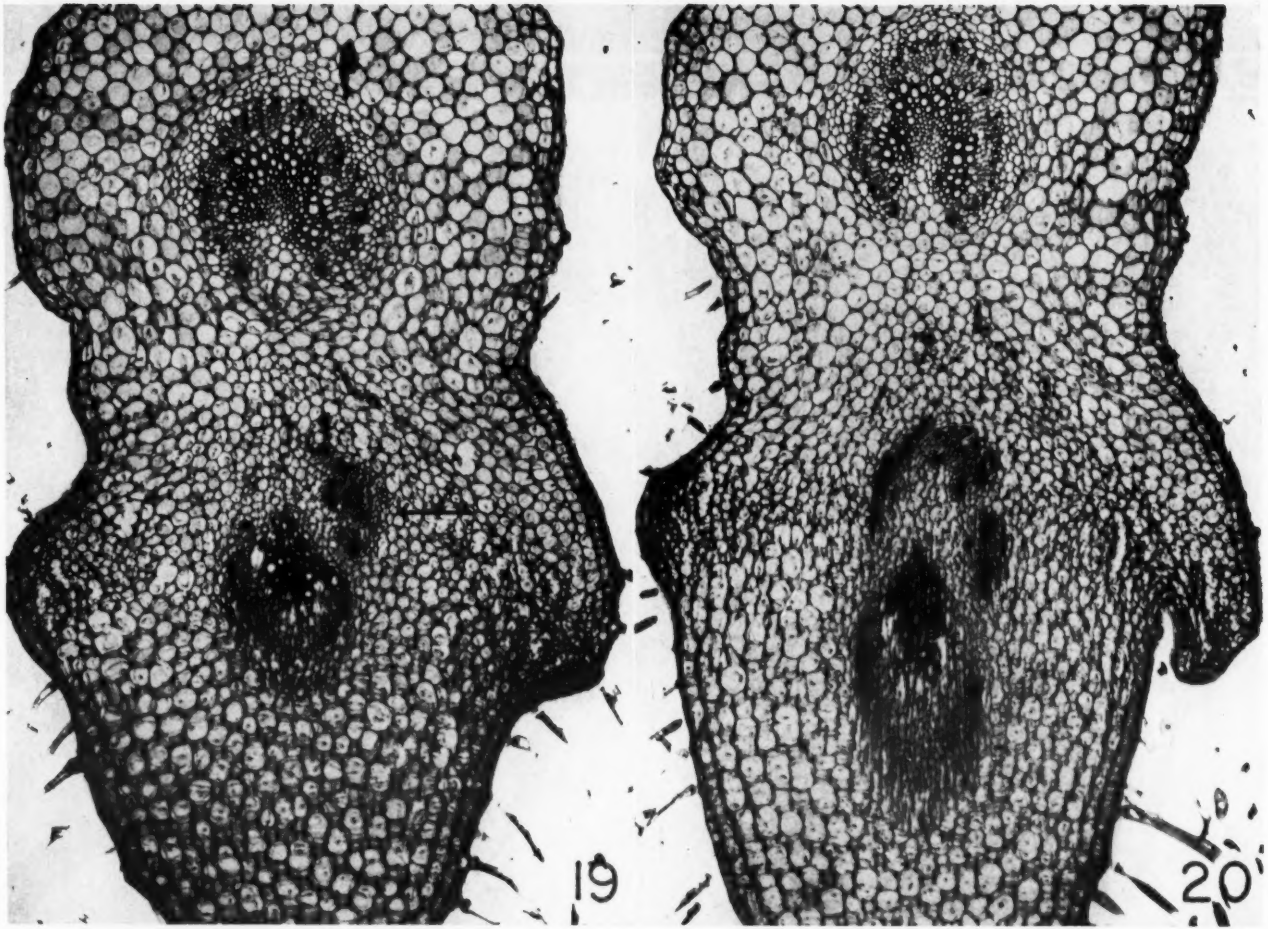

FIG. 19.—Transverse section of the thin shoot 8.6 mm. above base. The ring of bundles (arrow) is further developed. 65×. FIG. 20.—Transverse section of the thin shoot 8.8 mm. above base. The eustele, incorporating the trace of the second leaf, constitutes a ring of bundles. 65×.

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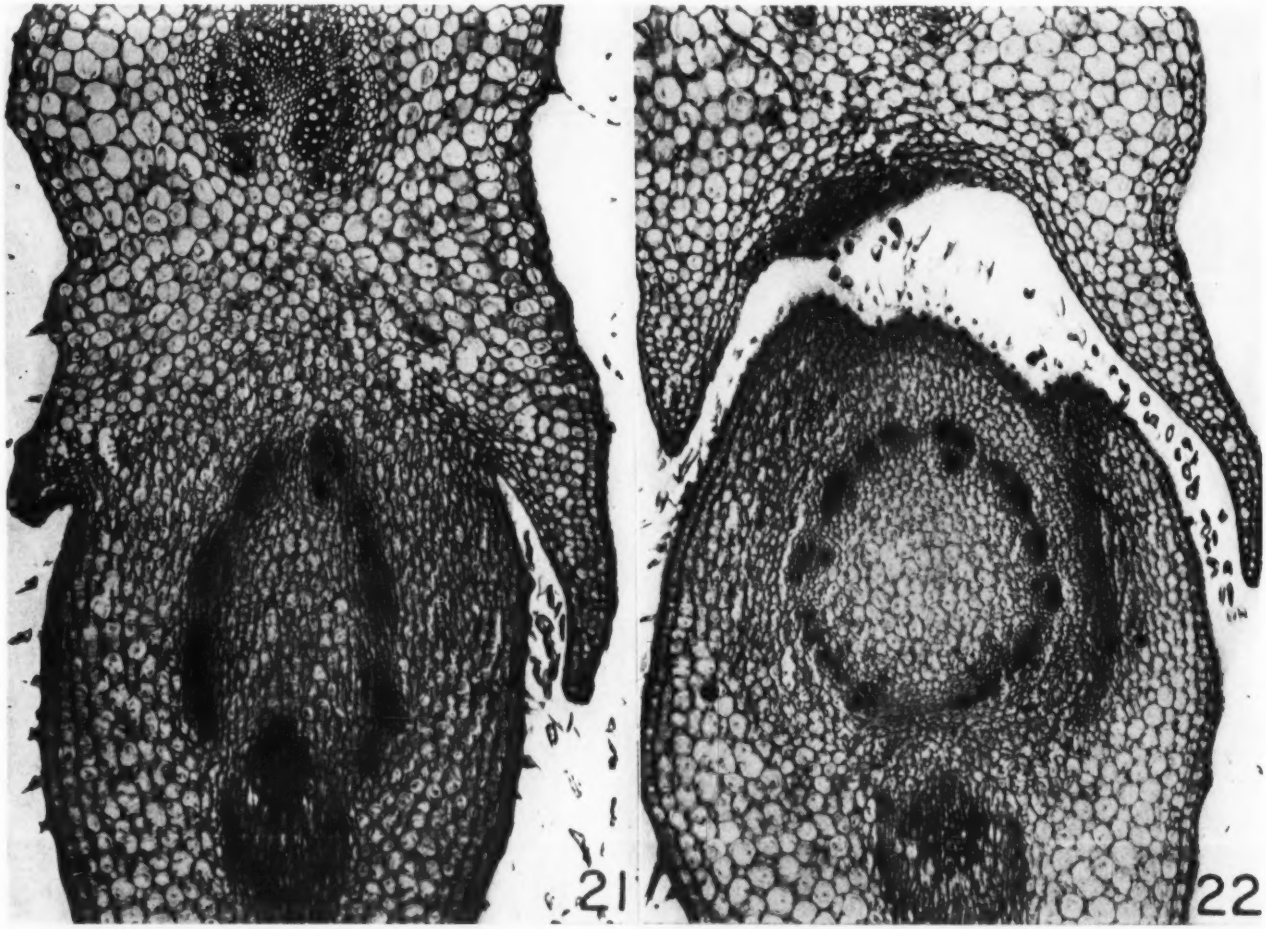

FIG. 21.—Transverse section of the thin shoot 8.9 mm. above base. The eustele has now a symmetrical form. 65×. FIG. 22.—Transverse section of the thin shoot 9.2 mm. above base. The eustele is symmetrical and constitutes a circle, in transection, of bundles surrounding a wide pith. 65×.

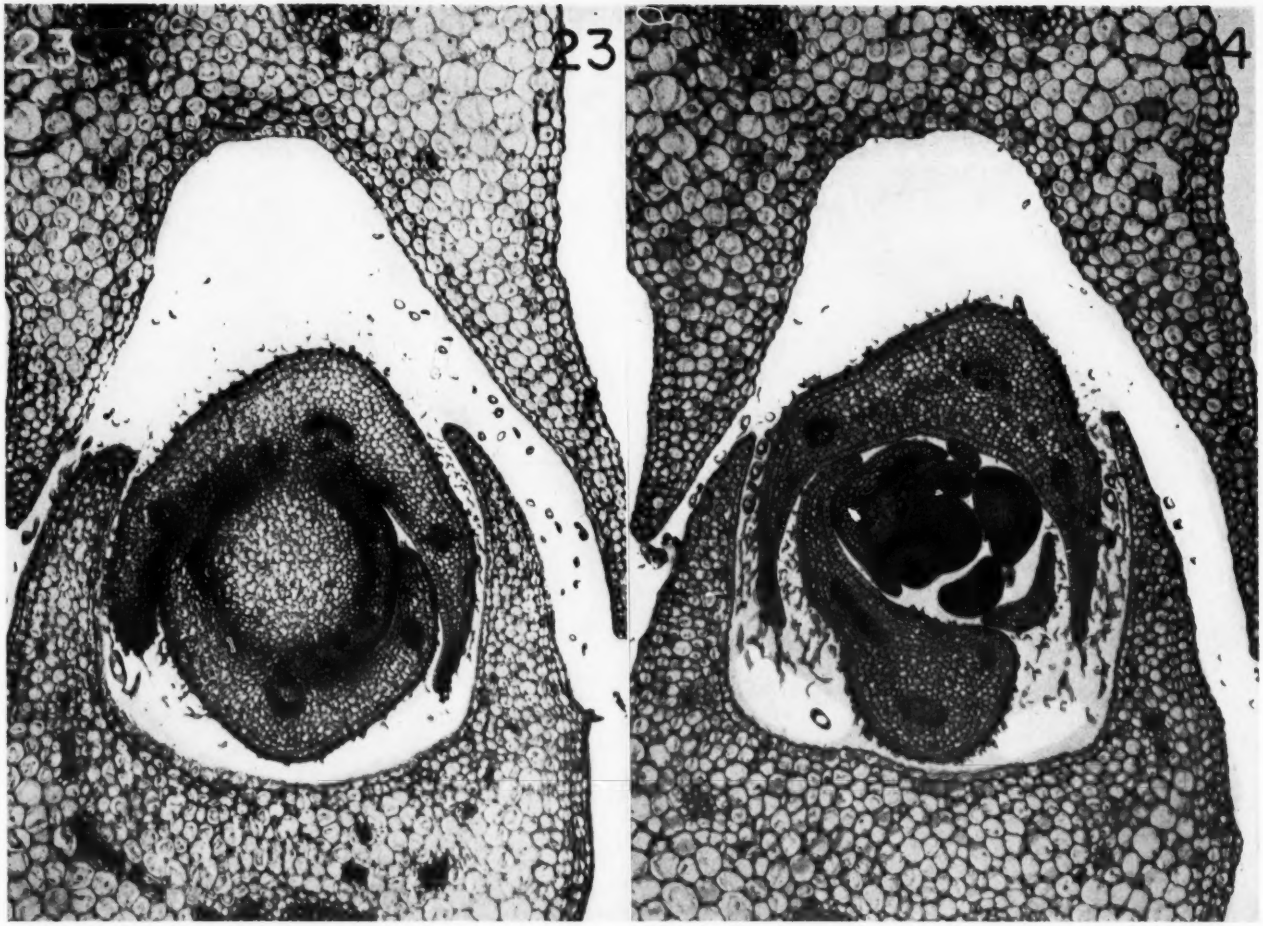

FIG. 23.—Transverse section of the thin shoot 9.5 mm. above base. The bases of the third and fourth leaves are seen attached to the stem. 65×.
FIG. 24.—Transverse section of the thin shoot 9.7 mm. above base, at the level of the shoot apex. The last five leaves around the shoot apex

1939). The formation of a bud by the divisions of a single epidermal cell in flax (Link and Eggers, 1946) represents the ultimate regenerative ability of a cell. It is interesting that there a mature parenchymatous cell had greater regenerative capacity than that found in the meristematic cells of the shoot apex of *Lupinus* by the present writer. In this direction the mature parenchymatous cells might be considered unspecialized, while the meristematic cells of the apex might be considered specialized.

Growth of shoot apices from small lateral portions of an apex has further demonstrated that there is no irrevocable destiny of these lateral cells, i.e., to form foliar primordia. Under proper stimulus (isolation plus destruction of the initials of the original apex) these flank cells regenerated into functional apices. A logical interpretation of this phenomenon is that the shoot apex determines what the individual cells do and not the reverse. The present results give experimental support to Hofmeister's (1876, p. 129) statements: "Das Wachsthum der einzelnen Zellen eines Vegetationspunktes ist geregelt und bedingt durch die, nach Erweiterung oder Erreichung bestimmter Formen hinstrebende Massenzunahme des gesammten Vegetationspunktes. Diese Massenzunahme kann nicht als die Summe der den einzelnen Zellen innewohenden individuellen Bildungstriebe aufgefasst werden. Denn es erfolgen, wenn durch äussere Einflüsse Gestalt und Entwickelungsrichtungen des Vegetationspunktes modificirt werden, Grossenzunahme und Formänderung in den einzelnen Zellen nur in diejenigen Maase, welches die allgemeine Wachsthumsrichtung des Vegetationspunktes den einzelnen Zellen giebt. Die Bildung neuer Zellen im Vegetationspunkte ist somit eine Funktion des allgemeinen Wachsthums, nicht seine Ursache."

The formation of shoot apices from the minimum number of cells by dividing the shoot apex into six parts has constituted a usable technique in studying the differentiation of tissues subjacent to the regenerating shoot apices. The two types of stele, tube and rod, produced by these small shoot apices are ones never found normally in this plant, but are normal to plants such as lycopods and ferns which are much lower in the evolutionary scale. Further, the formation of shoot apices from the lateral, leaf producing zones of the original apex demonstrates the latent potentiality of this meristem. The formation of organs by the shoot apex of this plant is thus not specifically pre-determined by heredity. These results are similar to Ward-

law's (1949) experiments in which leaves were induced to form in bud positions and buds to form in leaf positions by operations on the shoot apex of *Dryopteris aristata*. The genetic constitution of *Lupinus* obviously must be of such latitude as to permit these developments. The formation of organs can be regarded as primarily controlled by the mechanics of growth in the shoot apex.

It may be inferred from these data that the types of vascular tissues produced behind the apex of this plant have a relationship to the physiology of the shoot apex. Possibly this relationship is a nutritional one. The small neck of tissue connecting the smallest regenerating shoot apices to the subjacent vascular tissues might permit only sufficient nutrient to reach the meristem to form a small apex which could only determine a rod of procambium instead of the usual ring of procambial bundles enclosing a pith. Another explanation for the formation of a rod of procambium is that the functional shoot apices have been produced from a smaller number of cells than is normal to this plant. If the stimuli of procambialization that presumably move downward from the meristematic cells of the normal-sized shoot apex cause the formation of this provascular tissue as a ring of bundles, a reduction in size of the shoot apex might bring about a restriction of these stimuli to such a small area that a rod of procambium results. Wardlaw, by a series of experiments upon the shoot apices of ferns, caused the terminal meristem to form steles of less than normal complexity—either solenosteles or protosteles in plants that normally formed dictyosteles. The removal of foliar primordia (Wardlaw, 1944, 1946a) from a fern apex led to the attenuation of the shoot and to the formation of a subjacent solenostele; when primordia were again allowed to form on the apex the subjacent vascular tissue became a normal dictyostele. Wardlaw (1948) concluded that "Active shoot growth is usually productive of an undeniable cauline stele; on the other hand, active leaf development and relative inactivity at the shoot apex may be productive, at least for a time, of a vascular system which will appear to be a composite structure consisting largely of decurrent leaf traces with little or no cauline component." Application of this technique to *Angiopteris* (Wardlaw, 1946b) gave attenuated shoots in which the vascular systems were successively reduced from dictyostele to solenostele to protostele. The isolation of the central part of the shoot apex of Dryopteris (Wardlaw, 1947) by four longitudinal cuts, thereby

creating a shoot apex of much reduced diameter, allowed the formation of an attenuated shoot in which the subjacent vascular system was a solenostele. The narrow isolation (150μ) of the central part of the shoot apex of *Lupinus* (Ball, 1952) led to the formation of mature subjacent vascular tissues in the form of an almost interrupted cylinder, instead of the normal ring of vascular bundles or eustele. Some comparisons may be drawn between Wardlaw's results and the present work. It has been shown here that the small shoot apex in the first stages of its growth, before production of leaves, left behind a solid rod of procambium; this matured as a tube or rod of vascular tissues and constituted upon maturity the basal vascular strand of the thin shoot. The last stages of growth of the small shoot apex entailed leaf formation and the production of an eustele. Since the rod of procambium was formed by the regenerating small shoot apex before foliar primordia were present, and when the latter were formed by the apex in a normal manner the procambial pattern was changed to "bundles in a ring", it can be assumed that the rod of procambium represented the cauline vascular tissues.

Acropetal differentiation of procambium is described by Esau (1942, 1943) in the shoot apex of *Linum perenne*. This provascular tissue was considered possibly to begin differentiation under the site of a foliar primordium before the latter became evident. A similar conclusion was reached by Priestley, Scott and Gillett (1935) for *Alstroemeria,* Priestley and Scott (1937) for *Alstroemeria* and *Tradescantia*. Dixon (1936) thought that the already differentiated procambium might precipitate procambium from the undifferentiated meristem. Bower (1930) thought that the subjacent, mature tissues exerted an effect upon the shoot apex to cause the latter to produce a stelar structure suitable to the size ultimately to be attained. These descriptions and hypotheses of acropetal differentiation of procambium are not supported by the results of the present experiments. In *Lupinus* the formation of a suspended rod of procambium beneath the regenerated small shoot apex was apparently determined by the terminal meristem and not by the already differentiated subjacent tissues. The rod of procambium was a distinctive form quite unlike the previously existing subjacent provascular tissue and it is difficult to assume that the original vascular tissue, a ring of strands of procambium, would determine a rod in the acropetal direction. And finally,

the rod of procambium did not determine the shoot apex to produce an acropetal continuation of provascular tissues in the same form, but in the late stages of growth after leaves were formed the subjacent procambium was again a ring of strands with a wide pith. The rod of procambium was, at the earliest recognizable stage, present immediately beneath the small shoot apex, and was not attached to the subjacent pre-operation procambium. The first procambium formed by the small shoot apices was therefore a suspended strand. It is a valid interpretation, since it was not originally continuous with the subjacent procambium, that the rod of procambium differentiated basipetally from, and under the control of the shoot apex. Furthermore, the formation of leaves on the small shoot apex was not determined by the subjacent procambium, since the first leaves were formed laterally on the terminal meristem that was above a centrally-located rod of procambium.

The production at first by the regenerated small shoot apices of steles in the form of tubes or rods and later of eusteles may be considered evidence that such terminal meristems recovered their normal behavior during the course of prolonged growth. The increase in diameter of the apex after the production of the eustele is also evidence of recovery. Such activity may be used to support the idea that the shoot apex is not appreciably influenced by the type of subjacent vascular tissues that have already been produced by it. The tubes or rods of vascular tissues did not influence the shoot apices to continue to produce vascular tracts of these distinctive types. Instead, the shoot apex eventually began to produce the eustele characteristic of this plant. The hypothesis of independence of the shoot apex in morphogenesis of tissues has received support by these experiments. These findings are in agreement with the earlier results of Ball (1944) where shoot apices of *Tropaeolum* treated with growth regulators at first showed abnormal phyllotaxy and the occasional production of multiple leaves, but eventually returned to normal growth. Snow (1942) showed that shoot apices of some decussate plants were self-regulating in their phyllotaxy and that longitudinal splits may occasion only a temporary change to spiral phyllotaxis.

Summary

Shoot apices were divided into six approximately equal parts by longitudinal cuts 100μ deep. Unavoidable inequalities in these sectors

made them regenerate in different ways. Some sectors were above the minimum cell number for formation of an apex and grew quickly into normal apices with a subjacent tube of procambium. Other sectors were apparently at about the minimum cell number for formation of an apex. Previous to leaf-production, these small apices produced thin shoots having a rod of subjacent procambium. This provascular tissue matured into either a tube or a rod of vascular tissues. After the small shoot apices produced leaves, the subjacent procambium was produced in the normal manner, an eustele or ring of bundles surrounding a wide pith. The tube or rod of vascular tissues produced by the small apex appeared to be cauline vascular tissue; correspondingly, the eustele appeared to be composed of leaf traces. The formation of new shoot apices from lateral parts of an original apex—from parts that would have normally produced foliar primordia—was considered evidence that the formation of organs by this meristem is not specifically pre-determined by heredity. An individual cell appears thus to be relatively unimportant. A minimum number of meristematic cells must work together to constitute an apex. These meristematic cells are regulated in their growth by the apex as a whole, and a cell can change its function and activity if its relative position is changed by external circumstances, as for instance, by this operation. The meristematic cells of this apex appear to have probably less potentiality for regeneration than do certain large, mature parenchymatous cells. The regenerating shoot apex appears to be independent of the previously existing vascular system in its production of new vascular tissues and of new leaves.

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WEIGHT AND LENGTH MEASUREMENTS OF FETUSES OF KARAKUL SHEEP AND OF GOATS

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There seems to be a lack of extensive information on the prenatal growth of sheep and goats, especially of the latter. Apparently, the most extensive study of sheep fetuses is that of Cloete (1). He has reviewed most of the previous literature on the subject, and in his own experimental work has added much information on the enlargement of the uterus and the changes in various glands of the ewe during pregnancy.

Most data for the age-weight relationship in Merino fetuses give a logarithmic curve. There is considerable fluctuation in the weight of the fetus after 100 days, and weight is more variable than fetus length. The most reliable length measurement seems to be vertebral column length. Straight crown-rump length is nearly as satisfactory. The size of the fetus seems to be related to the size of the ewe, large ewes producing large lambs. Older ewes also tend to produce larger lambs.

Cloete has given data on the growth of different body regions, as head, trunk, limbs and tail. All but the head have similar curves, one of the second degree.

Data on goat fetuses seem to be lacking.

The present study was made to furnish additional data on the fetal growth of sheep and goats, and for comparison with other data available.

MATERIAL AND METHODS

The animals used in this study were Karakul sheep and Toggenburg and common American goats, surplus to the regular breeding experimental projects with these animals. The animals were bred to furnish fetuses of different ages for study of hair development by the animal fiber laboratory, and the same material was made available for fetal growth studies.*

* Acknowledgment is made to Miss Ethel H. Dolnick and Mrs. L. M. Hansen of the animal fiber laboratory for sharing the material and for assistance in collecting the data.

As soon as the fetuses were removed from the uterus they were wiped as dry as possible and laid out on the table with the back in as nearly a straight line as possible, care being taken to avoid stretching of the fetus. The distance from the crown of the head to the base of the tail was determined. Weight was also taken. Different types of curves were tried and the ones that fitted the data most closely were finally selected. This was determined by the lowest value of chi square, chi square being the sums of the ratio between the calculated measurement at a given age and the square of the deviation of this measurement from the actual measurement observed. Second degree curves, using actual weight and length rather than logarithms of these measurements, gave the closest fit. However, for weight of Karakul fetuses, a single curve did not give a very close fit. The single curve giving the closest fit was one of the fourth degree, but the values for ages below 71 days were considerably out of agreement. By using two segments of second degree curves having different constants, the data fitted closer, the break occurring between 71 and 80 days.

Results

Karakuls

The actual weight and length measurements along with the calculated values, and age and weight data of the dams are shown in Table 1. Since the number of animals available was small, there is only one observation for a designated age except for 45, 100 and 121 days. The latter two cases were twins. Length measurements of the 27 and 39 day fetuses were omitted from the calculations because of the inaccuracy due to curvature of the fetuses at these early stages. The calculated curve values did not deviate significantly from the actual fetus lengths at the various ages.

For fetus weight there seems to be two periods of growth governed by different growth rates. The first period ends between 71 and 80 days.* If the calculated curve for the 7 weights below 80 days is extended to 80 days, the calculated value for this age is 137 grams below its actual value. By calculation of the weights above 70 days as a separate curve, the calculated value for 80 days differs by only 35 grams from the actual value. The value of chi square was less

*The animal fiber laboratory has pointed out that hair anlage are present at this period. Whether this has any relation to the growth rate of the fetus at this time is not known. More fetuses around this age might eliminate the break in the curve if no relation to growth exists.

TABLE 1

MEASUREMENTS OF KARAKUL FETUSES WITH CALCULATED CURVE VALUES AND AGE AND WEIGHT OF DAMS

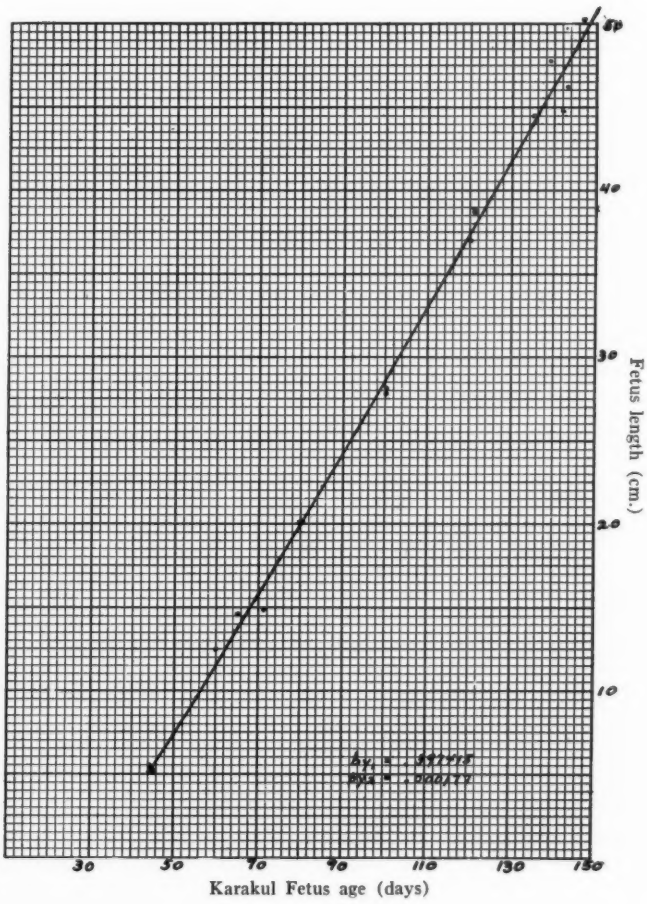
Dam				*Fetus*				
Number	Age (years)	Weight (pounds)	Sex	Age (days)	Weight (grams)	Length (cm.)	Calculated weight	Calculated length
KC 314	6	148	—	27	0.9	1.4	1.2	—
K 622	2	97	—	39	3.5	3.2	.4	—
KC 232	7	115	F	45	8.5	5.2	10.2	5.4
KB 332	6	92	M	45	8.6	5.3	10.2	5.4
KC 272	7	118	M	60	58.8	12.5	64.5	11.6
KC 249	8	127	F	65	103.0	14.6	92.1	13.7
KN 115	4	81	F	71	126.9	14.9	131.4	16.3
K 334	10	108	M	80	340.2	20.0	305.8	20.1
KC 198	9	110	M	100	861.8	28.2	907.0	28.7
			M	100	907.2	27.9		
K 412	8	81	M	120	1814.4	37.0	2040.4	37.4
KC 199	9	127	F	121	2131.9	38.9	2111.0	37.8
			F	121	2177.2	38.7		
KN 103	5	104	F	135	3674.1	44.5	3239.7	44.0
KN 45	7	90	F	139	3583.4	47.7	3610.1	45.8
K 495	6	118	F	142	3810.2	44.8	3901.9	47.1
K 451	7	112	F	143	3810.2	46.2	4001.8	47.6
K 617	3	106	M	147	4445.2	50.3	4414.8	49.4

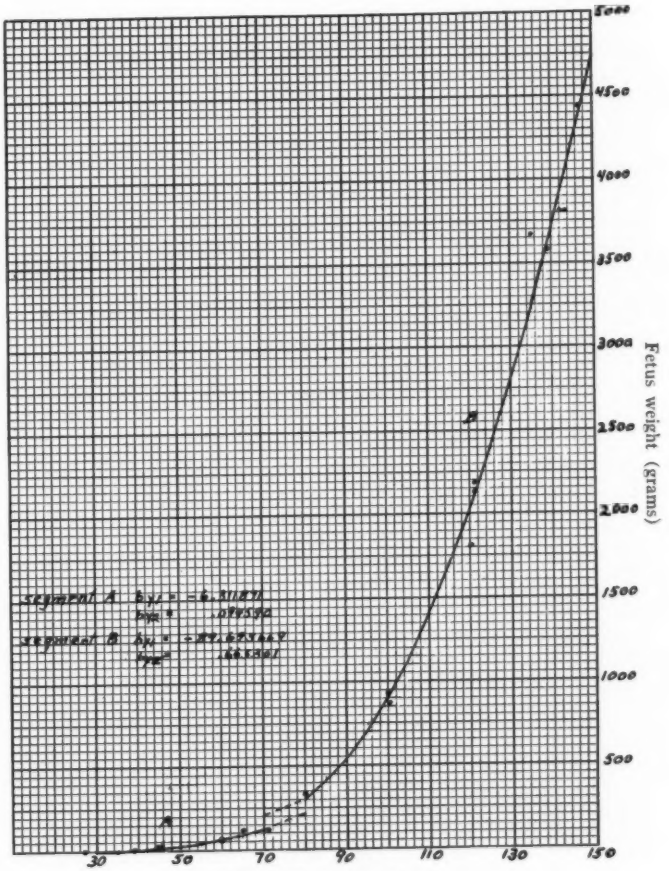
by using the two segments of second degree curves than for a single curve of the second degree or a curve of the fourth degree which had the second lowest value for chi square.

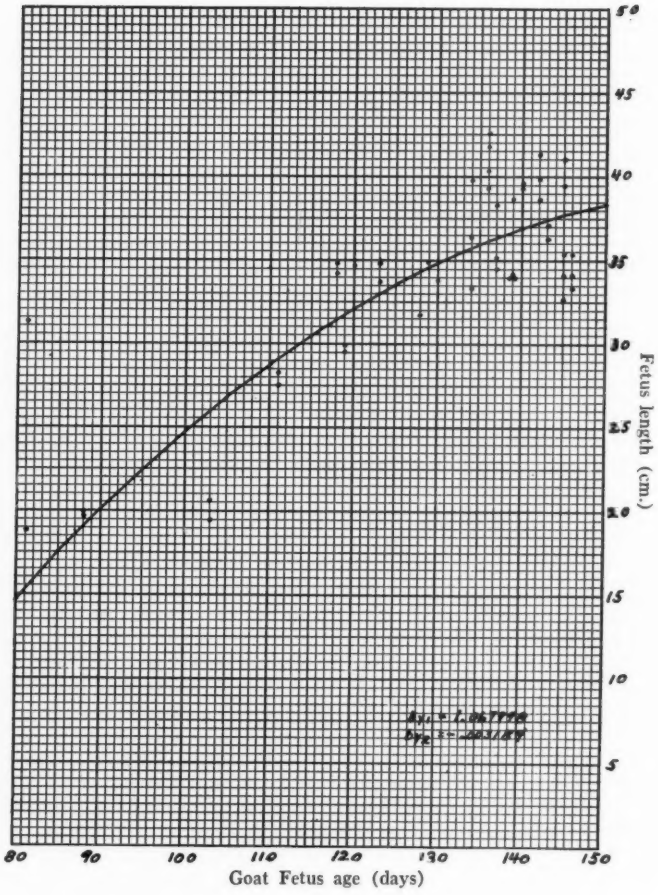
Curves for fetus length and fetus weight with the values of the constants for second degree curves are shown in Figures 1 and 2.

Goats

In the series of goat fetuses there were only 2 under 80 days of age, one each at 34, and 43 days. Only those above 80 days of age were used in calculating the curve values. There were several fetuses of the same age due to the occurrence of twins and triplets and more does bred for fetuses of definite age. The second degree curve fitted the data very well both for fetus length and weight. Weight measurements were left in pounds instead of being converted to grams as was done in the case of the Karakul fetuses. The goat data are shown in Table 2 and the curves for fetus length and weight with their constants in Figures 3 and 4.


50
40
30
20
10
30
50
70
90
110
130
150
Fetus length (cm.)
Karakul Fetus age (days)


5000
4500
4000
3500
3000
2500
2000
1500
1000
500
Fetus weight (grams)
30
50
70
90
110
130
150
Karakul Fetus age (days)


50
45
40
35
30
25
20
15
10
5
Fetus length (cm.)
80
90
100
110
120
130
140
150
Goat Fetus age (days)

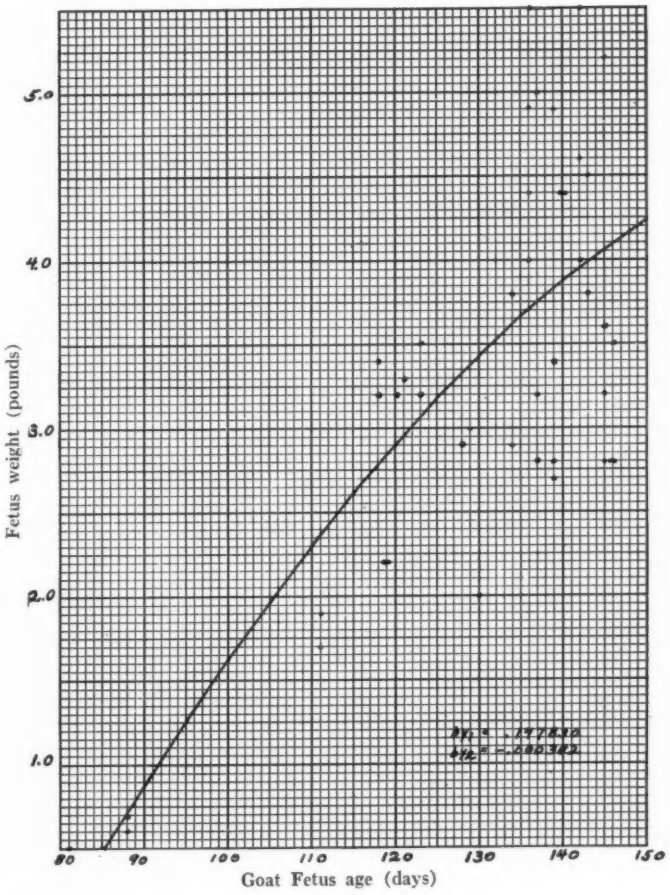

5.0
4.0
3.0
2.0
1.0
Fetus weight (pounds)
80
90
100
110
120
130
140
150
Goat Fetus age (days)

TABLE 2
MEASUREMENTS OF GOAT FETUSES WITH CALCULATED CURVE VALUES AND AGE AND WEIGHT OF DAMS

Dam				*Fetus*					
No.	Breeding*	Age (years)	Weight (pounds)	Sex	Age (days)	Weight (pounds)	Length (cm.)	Calculated weight	Calculated length
4	C	7	102	—	34	—	2.2	—	—
170	C	1	58	—	43	—	3.0	—	—
673	T	7	120	M	81	0.5	18.8	.13	15.4
13	C	6	96	M	88	0.7	19.9	.72	19.1
15	C	7	91	F	88	0.6	20.0	.72	19.1
244	T	10	—	M	103	—	19.5	1.84	26.0
				M	103	—	20.6	1.84	26.0
51	C	4	82	M	111	1.9	28.3	2.37	29.1
				F	111	1.7	27.5	2.37	29.1
156	T	1	97	M	118	3.2	34.2	2.79	31.4
				M	118	3.4	34.8	2.79	31.4
61	C	3	76	M	119	2.2	29.5	2.85	31.8
				M	119	2.2	29.9	2.85	31.8
154	C	1	67	M	120	3.2	34.7	2.90	32.1
9	T	8	76	M	121	3.3	—	2.96	32.4
26	T	4	110	M	123	3.2	33.7	3.06	33.0
				M	123	3.5	34.9	3.06	33.0
183	C	1	74	F	128	2.9	31.7	3.33	34.3
172	T	1	57	M	129	3.0	34.9	3.38	34.6
123	C	2	76	F	130	2.0	28.8	3.43	34.8
182	C	1	71	F	134	3.8	36.3	3.61	35.7
				M	134	2.9	33.4	3.61	35.7
73	C	1	—	M	134	—	39.8	3.61	35.7
86	T	2	83	F	136	5.5	42.5	3.70	36.1
				F	136	4.9	41.7	3.70	36.1
26A	T	5	85	M	136	4.4	40.3	3.70	36.1
				M	136	4.0	39.4	3.70	36.1
101	C	2	115	M	137	5.0	38.4	3.74	36.3
204	C	1	68	M	137	3.2	35.1	3.74	36.3
				F	137	2.8	34.5	3.74	36.3
54	C	4	80	F	139	2.8	34.0	3.83	36.7
				M	139	3.4	34.0	3.83	36.7
				M	139	2.7	34.1	3.83	36.7
5	C	7	90	M	139	4.9	38.7	3.83	36.7
20	C	7	98	M	140	4.4	39.6	3.88	36.9
				F	140	4.4	39.4	3.88	36.9
644	T	7	135	F	142	5.5	41.3	3.95	37.2
				F	142	4.6	39.9	3.95	37.2
				F	142	4.0	38.6	3.95	37.2
98	C	2	89	M	143	4.5	37.1	3.99	37.4
				F	143	3.8	36.3	3.99	37.4
2	C	5	84	F	145	2.8	32.6	4.06	37.7
				M	145	3.2	34.1	4.06	37.7
72	C	3	106	F	145	5.6	41.0	4.06	37.7
				F	145	5.2	39.5	4.06	37.7
45	C	4	80	M	145	3.6	35.4	4.06	37.7
19	C	6	76	F	146	2.8	34.1	4.10	37.8
				M	146	2.8	33.3	4.10	37.8
				F	146	3.5	35.4	4.10	37.8

* C—Common American; T—Toggenburg.

Comparisons

In order to make certain comparisons and correlations the weight and length measurements were corrected to a standard fetus age by means of the formula

$$Ys = Ya - (Xa - Xs)\ b_{y1.2} - (X_a^2 - X_s^2)\ b_{y2.1}$$

in which Ys is the standard value sought, Ya, the actual value observed at a given age, Xa, Xs and X_a^2 X_s^2 the actual and standard age and their square, b_{y1} and b_{y2} constants of the calculated curve. Karakul measurements were corrected to 100 days as the standard age and goats to 130 days, each being the approximate mean age of the fetuses for the two groups. The correction for Karakul weights was not entirely satisfactory for those ages under 80 days because of the difference in the constants for these portions of the curve. The weights for ages under 80 days were corrected to 80 days by the constants for the lower segment of the curve, then these 80-day corrections were corrected to 100 days by the constants for the upper segment of the curve. This is not an entirely satisfactory method, but the values obtained are in fair agreement with the values for the weights which were above 80 days.

Correlations between fetus weight and length were determined from actual values and found to be .947 for Karakuls and .955 for the goats.

There are several factors which might exert an influence on the results of the fetus measurements. Breeding is one of these. Since there were so few of the different Karakul crosses involved, no attempt has been made to classify them as to breeding. With the goats however, there are two distinct groups, those with Toggenburg blood, and the common Americans without Toggenburg influence so far as our history of the animals is concerned. For Toggenburg fetuses after correction to a constant age, the average weight was 4.006 pounds, for the common goats 3.154, and the difference was highly significant. The difference in fetus length also was highly significant, the mean values being 36.67 cm. for Toggenburgs and 33.89 cm. for common goats.

Age of dam correlations with fetus length and fetus weight were low for both Karakuls and the goats. Weight of dam correlations with fetus weight and fetus length were highly significant for the goats but not for the Karakuls. The correlation value for weight of

dam with fetus weight was .552 and with fetus length .498. Differences in fetus length and fetus weight due to sex were small and not statistically significant for either the Karakuls or the goats.

Variability of the actual measurements from the calculated measurements not only shows the agreement between the two, but also shows the uniformity or lack of uniformity in growth between the animals. Fetus length was more uniform in both Karakuls and goats than fetus weight. Coefficients of variability for fetus length were 3.71 and 9.47 for Karakuls and goats respectively, and for fetus weight 8.50 and 25.12 respectively. The coefficients of variability are about three times more varaible for goats than for Karakuls in both measurements. It was thought possible that the variation in age or weight of the dams might be partly responsible for fetus variation. The coefficient of variability in age of dams was 32.6 for the Karakuls and 61.6 for the goats. For dams' weight the variability was 15.8 and 20.9 for Karakuls and goats respectively.

The number of fetuses in the uterus might be expected to have a considerable effect on the weight and length of the individual fetuses. Twin fetuses of both Karakuls and goats weighed slightly more than single fetuses, and triplets in the goats weighed only slightly less. In no case were the differences in weight significant. There were no triplets found in the uteri of the Karakuls. Likewise fetus length did not differ significantly between singles, twins and triplets. Comparisons for Karakuls and goats are given in Table 3.

There were several fetuses of both goats and Karakuls of which the age was not known. From the weights and length measurements of these it was possible to estimate fairly accurately the approximate age. This was done according to the method described by Cloete (1). In most cases there was a discrepancy of one to four days in age between estimates based on weight and those based on length. In cases of twin or triplet pregnancies the mean fetus length and weight were used as the basis for calculation. The observed measurements and the calculated ages for fetuses of unknown age are shown in Table 4.

Discussion

In many respects the Karakul and goat fetus data agree with that of Cloete (1) for Merino fetuses. The Karakul fetuses in general were slightly shorter in crown-rump length and less in weight than the Merino fetuses for a given age. Diachkov *et al.* (2) found the

TABLE 3
COMPARISON OF KARAKUL AND GOAT FETUS DATA[1]

	Karakuls	Goats	
Correlations:			
Fetus length × weight	.947**	.955**	
Age dam × fetus length	.112	—.179	
Age dam × fetus weight	—.030	.056	
Weight dam × fetus length	.161	.498**	
Weight dam × fetus weight	.089	.552**	
Coefficients of variability:			
Fetus length	3.71	9.47	
Fetus weight	8.50	25.12	
Age of dams	32.62	61.58	
Weight of dams	15.81	20.87	
Averages:		Toggenburg	Common
Fetus weight		4.006 lbs.	3.154 lbs.
Fetus length		36.675 cm.	33.896 cm.
Length male fetuses	28.66 cm.	34.72 cm.	
Length female fetuses	28.64 cm.	34.91 cm.	
Weight male fetuses	847.15 gm.	3.40 lbs.	
Weight female fetuses	896.75 gm.	3.46 lbs.	
Effect on fetus length of:			
Singles	28.59 cm.	35.29 cm.	
Twins	28.84 cm.	35.00 cm.	
Triplets	——	33.62 cm.	
Effect on fetus weight of:			
Singles	853.01 gm.	3.47 lbs.	
Twins	917.50 gm.	3.55 lbs.	
Triplets	——	3.03 lbs.	

[1] Comparisons were made by correcting Karakul fetuses to a constant age of 100 days and goat fetuses to 130 days, except the fetus length-weight correlation and the coefficients of variability.
** Correlations significant at 1 percent level.

fetus weight of Karakuls, both with the ewes on pasture alone and those on pasture supplemented with grain, to be greater than in the present experiment. Fetuses at ages 60, 80, and 110 days agreed very well with the weight of Karakul fetuses of those ages reported by Nikolski (3). His 70-day fetus, however, was twice the weight of the present data, and his twin fetuses of 90 days were much lighter. Tänzer (5) gives the length of a 70-day Karakul fetus as 14.5 cm., varying only 0.4 cm. from the 71-day fetus of the present data.

Variability of 3.7 in fetus length of the Karakuls agrees well with that of Cloete who gives variability as less than 4 per cent. Variability of fetus weight, however, was considerably less, 8.5 as compared to about 20 per cent reported by Cloete.

TABLE 4
ESTIMATE OF AGE OF FETUSES FROM OBSERVED LENGTH AND WEIGHT MEASUREMENTS

Weight	Length	Estimated age (days)	
gm.	cm.	from weight	from length
Karakul fetuses			
1270	32.4	107	109
363	21.0	82	82
499	24.1	87	89
1088	30.5	103	104
771	27.3	96	97
1497	35.6	111	116
862	27.9	97	98
816	27.3		
1588	36.2	113	117
726	27.3	95	97
544	24.4	89	90
907	27.9	97	97
726	27.0		
862	29.2	98	101
2404	41.3	125	129
544	24.7	89	91
907	29.7	99	102
0.7	—	—	27?
0.7	1.5		
3130	44.6	134	136
998	31.3	101	106
136	14.8	72	68
1.8	2.3?	—	37?
2240	41.1	126	126
Goat fetuses			
—	25.1	—	101
—	17.1	—	84
3.4 lbs	34.1	130	128
3.2 "	33.7	125	127
5.1 "	40.6	171*	164*
—	7.3	80—	67
3.6 "	35.4	134	133

* Obviously this was an unusually large fetus and near term.

Robinson (4) states that the variability of fetuses is much greater during the last half of pregnancy during the period of maximum growth, than during the first half. He found a multiple pregnancy of six fetuses at 61 days of age which were within the limits for singles in weight and length measurements. At equal ages the goat fetuses were less in weight and in length than the Karakul fetuses. This is to be expected, however, as the weight at birth averages about six pounds and ten pounds respectively.

The age and weight variability of the Karakul dams appeared to have little influence on the length and weight of the fetuses. This is in part shown by the low correlation of age and weight of dam with

fetus age and weight. The mean age of the Karakul dams was 7.1 years with a range of two to ten years, and the weight range was 81 to 148 pounds with 107 the mean.

With the goats, however, there was a considerable effect of dam's weight on both fetus length and weight, although the variability of the dam's weight was only slightly greater than that of the Karakuls. While dam's age was highly variable, the effect on fetus weight and length was negligible. The range in dam's weight among the goats was 57-135 pounds with the mean 88.9 pounds. The mean age of the goat dams was 3.4 years with a range of one to ten years.

Summary

Weight and length measurements of Karakul and goat fetuses of different ages are given. The data are in close agreement with the work of others with these classes of animals. The goat fetuses at all ages are smaller than Karakul fetuses due to differences in the mature weight of the two species. The weight-length correlation for both species was nearly equal and exceeded 0.9.

Dam's age had little effect on fetus length or weight in either species for the ages of dams involved. Weight of the goat dams, however, had an appreciable effect on fetus length and weight. The coefficients of variability of weight and length of the goat fetuses were approximately three times as great as for the Karakul fetuses. This may be due to breeding, since the Karakuls were a more homogeneous group, so far as breeding was concerned, than the goats.

There was little difference in length and weight of the fetuses due to single, twin or triplet pregnancies.

From weight and length of fetuses, it is possible to estimate fetus age with a fair degree of accuracy, $\pm$ 2 to 4 days.

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THE PROBLEM OF DIRECTIVES TO CELLULAR PROLIFERATION AS ILLUSTRATED BY ONTOGENETIC PROCESSES IN CERTAIN FISHES

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Introduction

At the very beginning of life, the first primordial cell or its biochemical precursor was immediately confronted with the problem of its own internal organization and operation and, nextly, with organization and control of its own reduplicative efforts. This is practically the equivalent of saying life was only a highly limited possibility until these two problems were sufficiently solved. Implicit in these seemingly elementary statements is the idea that pre-living autocatalytic enzymes, or whatever precursor of life one's imagination prefers, had to "solve" the cancer problem before life in the ordinary sense could appear. How many hopeful starts which failed because growth was so unorganized as to be quickly self-destructive is an idle speculation, but this thought serves to underline the idea of the co-extensiveness of the cancer problem with life itself.

Uncontrolled growth (unorganized) certainly came before organization appeared, the possibility of inter-cellular organization arising with the first colonial protozoa or the early metazoans. If primitive cellular elements remained in contact the growth was "invasive" in nature. If the cellular elements became detached from the parent colony and moved away by their own motility or were transported passively by exterior mechanical forces to new sites where they established new colonies, they were in effect "metastasizing." In ecological terminology this is "distribution," "colonization" et cetera, while in cancer workers' terminology, it is "malignancy." It is thus evident that "invasion" and "metastases," the principal destructive mechanisms of tumor growth can appear only after an organism has developed to a level of differentiated and organized tissues. These two necessary resultants from uncontrolled growth differ only in that the first (ecological) is exterior to the parent being and the second (malignancy) is interior to the individual. See especially the views of Zahl (1948) on similar and related matters.

The various mechanisms which control growth, or if you prefer, prevent any and all growth from tumorous randomness have been extensively studied by a variety of disciplines such as experimental morphology, experimental embryology, physiology and biochemistry, while the mechanism of the inheritance of the minute detail of such control forms a greater part of genetics. For present purposes it is not necessary to consider the operation of these processes, but only to point out that what has been discovered in these fields indicates an amazingly complex and minutely detailed totality of organic activity directed specifically toward the precise control of the growth and development in all its varied aspects.

That the control is nevertheless not absolute is, of course, evidenced by abnormal growths which in many cases have been traced to known causes. For purposes here they may be conveniently grouped into three superficially distinct kinds, although each tumor necessarily partakes of each category.

Genetic. It has been abundantly shown that a variety of tumors are "heritable" in the Mendelian sense in the type of cell involved, its locus and time of appearance. Certain of these cases are apparently intrinsic, while others are evidently merely the inheritance of a lack of protection from various carcinogenic agents.

Environmental. It has likewise been abundantly shown that various environmental influences can induce tumors. Certain chemicals, mechanical irritation, hormones, radiation and even the absence of sufficient light, Rasquin and Rosenbloom (in press), has been shown to directly or indirectly induce such uncontrolled proliferation. Here the sensitivity of the individual to any one of these environmental agents is evidently primarily a matter of genetic constitution.

Spontaneous. Most, if not all, "spontaneous" tumors are probably so-called merely because of ignorance of their genesis. However, it is conceivable that certain tissues are foredoomed to proliferate randomly after a certain length of time as a basic necessity of the process of aging.

The preceding outline of current thoughts on the problem of the organic control of growth was given in the manner stated in order to more easily develop certain ideas concerned with the continual struggle, that has existed as long as life itself, between the directing of cellular multiplication into channels useful to the organism as a unit structure and the tendency of cells to proliferate independently

and destructively. Workers in the fields of cancer research and the innumerable related groups, endocrinologists, geneticists, physiologists, biochemists et cetera, are prone to think in terms of "normal" and "tumorous" individuals. This is convenient for most practical studies but is, as with most such matters, an oversimplification. Practically every individual has some cellular "abnormality"—a mole —a wen—to say nothing of interior blemishes about which we usually know nothing. These are evidently either self-limiting or the organism has regained control and stopped further proliferation. Lucké and Schlumberger (1949) in considering related matters wrote, "There is no sharp dividing line between 'true' neoplasms and certain exaggerated forms of hyperplasia. Indeed the latter may merge by ill-defined stages with the former," and in connection with thyroid "tumors" the following: "They will raise the question of the relation between long persistent hyperplasia, which in some stages is reversible, and the development of non-reversible neoplasia." The latter view would indicate a temporary failure of whatever control mechanism is at the locus of the new growth.

If these views are sound and if there is in fact such a struggle between the organism's integrity and random cellular proliferation, with occasional slips of the control mechanism, greater evidence of such a situation than an occasional mole or similar item would be expected not only in ontogeny but in phylogeny as well. It is the chief purpose of this communication to point out that such cases do exist and to suggest that certain structures which are now recognized as part of the normal development of specific organisms may in fact have started as a "tumor," which instead of killing, became controlled, not at the cellular level but at the tissue level. This subsequently became reintegrated into the organism's economy as a whole, but left telltale evidences of its dangerous origin. Such an event would naturally be in the nature of an evolutionary change of some magnitude.

Illustrations

Specific instances illustrative of the preceding views follow. Fishes have been selected for this purpose simply because they constitute the animals with which the author has greatest familiarity. A worker in another group could doubtless find equally good or perhaps better examples in his area of interest.

The "ornamental" overgrowth of the so-called Lion-headed gold-

fish, a strain of *Carassius auratus* (Linnaeus), which was developed by the Chinese and Japanese by selective breeding has been studied by Matsui (1925). Although it evidently arose as a product of human selection instead of natural selection, it would seem to have relationship to epithelioma or papilloma. Such a growth may or may not have some utilitarian value to the individual or species. At least it must not be destructive to either the individual or the strain except that such growths could become destructive without detriment to the strain after the biological utility of the individual had ended. The dermal overgrowth above noted is not erratic as to size or locus and is definitely integrated as a regular part of the individual. Related strains of goldfish, evidently carrying more or less of some of the genes involved, occasionally do develop a similar overgrowth of lesser size and of an unsymmetrical nature both as to shape and location.

Pygidium totae Miles is so encircled by six or seven fatty rings that the natives of Colombia, where the fish is found, sometimes use it as a candle for illuminating purposes. Schlumberger and Lucké (1948) found the accumulation to be made of adult fat cells. It is not clear at this writing whether this should be considered a normal condition or a lipodystrophy spread through an entire population. It is noteworthy though that these bands of fat evidently follow the metameral arrangement of the underlying segmented body muscle.[1]

It has been shown by Breder and Rasquin (1951) that the marked change in shape of the dorsal fin of *Tylosurus raphidoma* (Ranzani) from juvenile to adult takes place very rapidly and does not involve the resorption of material as in most such ontogenetic changes. The area of the fin destined to disappear becomes an area of intense melanosis. This then breaks down and sloughs away. While this is taking place, a reduction of mandibular appendages goes on simultaneously, accomplished by the more usual resorption method.

As *Chaetodipterus faber* (Broussonet) ages, a great bulbous swelling of the first few interhaemals of the anal fin takes place, accompanied by various osseous overgrowths on the skull. Lucké (1948) con-

[1] An attempt was made to obtain living representatives of this species for further study, but communications with its discoverer, Mr. Cecil Miles, indicated that such was not practicable. He indicated that unlike other pygidiids, these are deep water fishes living at depths of perhaps not less than 100 or 150 feet. The only examples which have been collected were washed ashore in a moribund or dead condition. Furthermore, since steelhead trout have been introduced into these waters it is some years since any have been seen at all.

sidered these as a hyperostosis. Actually they are a perfectly normal development that all individuals show when of a sufficient size and age. No individuals, seen by us over a long period of years, that exceed 18″ in length have failed to show this condition. There is but scant evidence of it in smaller individuals. If these growths were present in only occasional individuals they would probably be considered osteomas. Such growths generally are so considered in other species, as in *Pagrus major* (Schlegel) described first by Takahashi (1929).

These overgrowths in *Chaetodipterus* have been known for long by fish osteologists. For example, Gregory (1933) figured such a skull and wrote in connection with a discussion of the balance and flotation of fishes that "In *Chaetodipterus faber*, for example, the great supraoccipital crest and interorbital bridge are much swollen and very dense, so that they must measurably tend to depress the forepart of the fish and perhaps require correctional movements of the pectoral fins or counterbalancing in some other parts, as in the swollen epineural and interhaemal spines." Gopinath (1952) extends this view to include rather similar looking overgrowths on the skulls of three carangin fishes, *Caranx sexfasciatus* Quoy and Gaimard, *Alectis ciliaris* (Bloch) and *A. indica* (Ruppell). He wrote that ". . . only fish above 18 inches in length did show this formation" which is in agreement with our measurements on *Chaetodipterus*. It would be tempting to assume that there is something about a fish of such a length that called for this sort of overgrowth, perhaps on some such mechanical basis as has been noted by these authors. While, of course, every structure and its position has a direct and immediate effect on the flotation of a fish it by no means can be assumed that bony growths are *ever* added to a fish as a response to needs of equilibrium. Other compensating mechanisms are available to fishes that protect it to a very great measure against just such loading or "shifting of cargo." The size of a meal which a fish may swallow, or the great loss of bulk a female fish may suffer on spawning, indicate what large and sudden changes of this sort a fish may pass through without interference with its flotation or equilibrium. Furthermore, these bones are packed with fat and have little difference in specific gravity from that of muscle which they may displace. Also many fishes show no such changes with age and evidently the carangins described by Gopinath (1952) showed no "compensating" swelling of the interhaemal spines as in *Chaetodipterus*.

In their list of reports of osteomas or hyperostosis in fishes Schlumberger and Lucké (1948) indicate 15 genera of teleosts suffering from various forms of such conditions. These are widely scattered including both acanthopterygians and preacanthopterygians. Six of the genera show modifications of the processes of the axial skeleton, involving the haemal or neural spines of the interhaemals. Four major groups of fishes are represented by these six, as follows:

Percoidea	
Sparidae	*Pagrus major* (Schlegel)
Heterosomata	
Hippoglossidae	*Paralichthys olivaceus* (Temminck and Schlegel)
	Psetta maeotica (Pallas)
Squamipinnes	
Chaetodipteridae	*Chaetodipterus faber* (Broussonet)
	Platax pinnatus (Linnaeus)
Plectognathi	
Monacanthidae	*Alutera schoepfi* (Walbaum)

The striking feature of these four groups of fishes is that, whatever their differences may be, they are notable as fishes of very considerable body depth with much flattening from side to side. Concomitant with the development of such a body form goes, of course, a very considerable elongation of the bones involved.

In a similar manner, osseous overgrowths on the cranium, may be considered from various sources. Gopinath (1952) discussed three carangins, Barnard (1948) one carangin and one sparid, and Ebina (1936) one sparid. The groups and species represented follow.

Scombroidei	
Carangidae	*Caranx sexfasciatus* Quoy and Gaimard
	Caranx equula Schlegel
	Alectis ciliaris (Bloch)
	Alectis indica (Ruppell)
Percoidea	
Sparidae	*Chrysoblephus gibbiceps* (Cuvier)
	Evynnis cardinalis (Lacépède)
Squamipinnes	
Chaetodipteridae	*Chaetodipterus faber* (Broussonet)

The striking feature of these fishes is that they are all provided with a very pronounced supraoccipital bone which extends upward from the roof of the skull as a thin median structure in the young before the overgrowth occurs. While most of these fishes are deep bodied the two *Caranx* of course cannot be considered in that category. Here again the overgrowth has occurred on a structure which has gone through notable evolutionary enlargement. It is noteworthy that Ebina found that while both sexes of *Evynnis* showed these overgrowths, the old males had them most pronounced.

No similar association could be found between any of the other conditions reported by Schlumberger and Lucké, either as to phylogeny, morphology or locus of the tumor. These all showed no tendency to be in the least symmetrical and in no way suggested that they might be under at least partial control or on the way to reintegration in the developmental scheme. Chabanaud (1926) recognized the widespread hyperostosis in several sciaenids as something which may be integrated with the metabolism of the species. In the earlier literature Köstler (1882) discusses similar matters in a variety of species and sites and gives the still earlier references now chiefly of historic interest.

Discussion

The introductory remarks and the illustrative examples which indicate clearly how difficult it is to always distinguish various types of tumors from stages in the normal ontogeny of certain fishes lead inevitably to the following considerations. The growths mentioned all have a marked amount of symmetry inherent in their formation and to this extent differ from the usually marked asymmetry of either benign or malignant growths which on that basis alone would not even figure in such considerations as the present. For example the papillomas on *Iridio bivittata* (Bloch) described and figured by Schlumberger and Lucké (1948), and with which we have some passing familiarity,[2] is clearly a proliferative process that develops with little or no reference to the "ground plan" of the creature and occurs in a variety of sites. It is clearly an adventitious development. The regularity and uniform locus of the cases under consideration are important matters in connection with the attempt to consider them

[2] There is a rather high incidence of this tumor in the fishes about the dock at the Lerner Marine Laboratory at Bimini, Bahamas, which is currently under study by Dr. R. F. Nigrelli of the New York Zoological Society.

either as neoplastic developments that are coming or have come under recontrol by the organizational system of the animal.

The point that such growths are under genetic control need hardly be labored, for in the cases mentioned they appear at regular times in the ontogeny of the individual. At the very least they have not been eliminated from the gene pattern. Actually one may prefer to consider them as well integrated into the chromosomal structure. Probably cases at either end of such a series could be found if it were possible to assay these things, perhaps after the manner of the studies of Gordon (e.g. 1937, 1941, 1948, 1951) on xiphophorin fishes. In this work the interaction of the inheritance of simple pigmentary pattern and malignancy has been worked out in often exquisite detail.

Some cases of fish tumors may have been instigated by external influences, such as trauma, suggested by Takahashi (1929) and Kazama (1924)) but doubted by Schlumberger and Lucké (1948). In any case in a situation of this kind and in others of a similar sort there must have been a genetic basis for the susceptibility for tumor growth under suitable stimulation.

Naturally such speculation leads to a consideration of the role of hormonal imbalance or of a slightly inadequate endocrine control. The relationship of such matters to mere hypertrophy of parts has never been considered in such connections, as for example the wattles of male birds or the supraorbital torus of male hominids which naturally come to mind.

Whether these over-extended tissues are benign or malignant would probably vary with the species and the nature of the structure. In the case of *Tylosurus* it would seem certainly to be benign—or is it simply self-limiting in the sense that the tissue breaks off because of structural weakness before invasion or a metastatic condition can develop—or has the fish developed, presumably on a selective basis, a mechanism which prevents dangerously destructive cellular activity and so reintegrates the detail into its normal growth pattern?

In the situation in *Chaetodipterus* and *Carassius* it would seem to make little difference whether the growths were benign or not for they appear relatively late in life and do not grow to any appreciable size until sexual maturity has existed long enough for considerable reproduction. It could be that these two fishes would be doomed to eventual death from these growths if they did not die of something

else first. This thought is in keeping with a variety of views on the fate of other tissues in animals generally if the creature is kept alive long enough. Here these cogitations become involved with general gerontology. If, by selection, an animal can postpone the onset of a tumor long enough and localize it appropriately, it would seem to make little difference to either the life of the species or the individual. Such a situation would seemingly involve only a gene or modifier which would appropriately delay the appearance of the neoplasm. Such genetic behavior is of course well established and known to occur. With such a view it would be practically impossible to say what characters in a senescent organism were of a tumorous basis and what were not.

If the factual data in the preceding remarks can be taken as evidence of a weaving in and out of organizational control or its lack, as part of the general evolutionary process, it is possible to develop some rather interesting thoughts. Considering a genetic drift of such growths through a population in relation to natural selection it would be easy to arrive at the conclusion that a certain amount of instability in the growth controlling mechanism might be of very great utility to the survival of the strain carrying it. No doubt, because of the primary medical genesis of cancer research, thinking has gone along the line that the widespread incidence and large variety of neoplasms, in all groups that have been at all well studied, is merely a failure of organisms to maintain at all times a full organic integrity. Evolutionists have talked for long about most mutations being deleterious. In an analogous manner we might talk about most tumors being deleterious if not downright fatal without in the least doubting that in an evolutionary sense such escapes from rigid control of growth designs may have been of large significance in the evolutionary deployment of organisms. This placing of tumor development as a part of the evolutionary apparatus relates, of course, to what Breder (1947) called a "principle of imperfection" both in regard to the maintenance of a definite degree of symmetry in organisms and in regard to the value of the absence of absolute perfection in animal behavior. Thus, failure to attain more than a minimal degree of control over niceties of symmetry, accuracy of behavior and coordination of all growth may actually have a more widespread and deeper lying survival significance than is generally recognized.

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A HISTOCHEMICAL STUDY OF CERTAIN ENZYMES IN THE "ADIPOSE TISSUE" OF THE CHICK AND MOUSE WITH SPECIAL REFERENCE TO EMBRYONIC STAGES[1,2]

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In a series of investigations Wassermann (1926, 1927, 1929, 1931, 1932, 1933) developed the hypothesis that the fat lobule is a reticuloendothelial type of organ, for he found that it arises from a syncytium formed from a ramified capillary network and adjacent mesenchyme. This complex of perivascular cells and capillaries possesses the character of an organ by virtue of its structural and functional organization, and hence, Wassermann designated the anlage of the fat lobules as primitive organs and the definitive structures as fat organs. This concept, together with the investigations of Schoenheimer (1946), who demonstrated the dynamic nature of fat storage and recurrent fat mobilization, has led to a renewed interest in the structure and physiology of the so-called "adipose tissue." For an excellent review of the recent literature, see Wertheimer and Shapiro (1948).

A number of investigators have utilized histochemical methods as a means of studying various enzymes and their possible role in the adipose tissue of newborn, young and adult animals but surprisingly little work has been done to determine when and where various enzymes develop in the fat organs of the embryos.

In adipose tissue of the rat, Hausberger and Neuenschwander-Lemmer (1939) discovered a relationship between glycogenesis and a rise in alkaline glycerophosphatase activity. Doyle (1948) detected slight alkaline phosphatase activity in adipose tissue of *Drosophila* sp., and Yao (1950a, 1950b) confirmed its presence in the larva of the same insect and discovered that there was strong alkaline phosphatase activity in the fat cells of the histolysing tissues of the pupa. Cater

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and Lawrie (1951) reported acid phosphatase and esterase activity in the adipose tissue of the chick at hatching but no definite indication of alkaline phosphatase was found.

Littrell (1948) reported phosphatases and lipase in the brown adipose tissue of the rat, while Baker *et al.* (1950) reported specifically that alkaline phosphatase was present in nuclei and cytoplasm of the same tissue. Fawcett (1952) found that alkaline phosphatase, esterase and glycogen were more abundant in the brown than in the white adipose tissue of the rat, and he confirmed the presence of glycogen in the brown adipose tissue of the mouse after feeding periods which had been reported previously by Micklewright (1950). In the embryonic chick the preen glands which are thought to supply oil to the feathers, were found to contain a high concentration of acid phosphatase (Cater and Lawrie, 1951).

Artom (1935) quoted Quagliarello and Scoz as having reported in white adipose tissue a true lipase which split triglycerides of the higher fatty acids. Renold and Marble (1950) corroborated this by demonstrating lipolytic activity in the subcutaneous adipose tissue of man and the rat, as well as in the brown fat bodies of the latter. Gomori (1946) succeeded in demonstrating lipase in the adipose tissue of the rabbit and rat, occasionally in the same tissue of man but not in the rhesus monkey, guinea pig or dog. Barrnett and Seligman (1951) detected esterase activity in the brown adipose tissue of the rat confirming the work of Fawcett (1947, 1949) and Littrell (1948). Hunter (1951) reported esterase activity in the "hibernating gland" or brown fat bodies of the 15 and/or 20 mm. stages of the mouse embryo but not in the 5 and 10 mm. stages. It should be pointed out that Gomori (1949) in evaluating his technique for demonstrating lipase activity with new substrates indicated that the use of "Tween 40" or "Tween 60" detected esterase, not true lipase, activity. Consequently the term esterase will be used as equivalent to the lipolytic enzyme which earlier workers, using the Gomori procedure, called lipase.

In view of the limited number of histochemical studies which have been made on embryonic adipose tissue, it is the object of this paper to present observations which have been made on the occurrence of acid and alkaline phosphatase, esterase, and glycogen in the subcutaneous fat bodies of chick embryos and newly-hatched chicks and in the interscapular brown fat bodies of embryonic and newborn mice.

Materials and Methods

White leghorn embryos from 13 to 21 days of incubation and chicks up to two days after hatching were killed and the subcutaneous fat bodies from the lateral cervical, lateral abdominal, lateral thoracic and sartorial regions were removed and placed in chilled acetone for fixing. Embryos of albino mice after 16 to 20 days from inception of gestation and young mice up to 6.5 days old were decapitated and the interscapular brown fat bodies were removed and similarly fixed. For comparison with fixed tissues fat bodies from both chicks and mice were frozen in 0.9% saline and kept in the refrigerator at —8° C. until the determinations were made. The histochemical procedures and improvements of Gomori (1941a, 1941b, 1949, 1950; see also Glick, 1949) were employed in the detection of acid and alkaline phosphatase and lipase with the necessary modifications for using frozen sections.

The citrate buffer, with which sections were treated before alkaline phosphatase determinations, was prepared according to Lillie (1948). For alkaline phosphatase determinations sections were incubated in a substrate medium of sodium alpha glycerophosphate at pH 9.0-9.6 for periods varying from 15 minutes to 24 hours, at 37° C. Control sections were incubated at the same time in a buffer medium without substrate, or in substrate medium with potassium cyanide as the inhibitor. For acid phosphatase determinations sections were incubated for from 15 minutes to 2 hours, in acetate buffer at pH 5 with glycerophosphate and lead nitrate. Controls were incubated simultaneously in buffer, or substrate with added sodium fluoride as inhibitor. Sections tested for esterase were incubated for 1 to 24 hours in maleate buffer at pH 7 with "Tween 40" or "Tween 60" and calcium chloride. Controls were incubated in the buffer medium without the "Tweens."

Tissues fixed in Bouin's and embedded in paraffin were sectioned at 7μ and tested for the presence of glycogen by the Periodic acid-Schiff's Reagent (McManus, 1948). Interscapular fat bodies of mouse embryos were tested from 16 days' gestation to term and from 1.5- and 3-day old young mice and from the adult. Fat bodies from chick embryos aged 14, 15, 17 and up to 20 days of incubation were tested also. Control sections were treated with potassium periodate instead of periodic acid or were incubated with human saliva before the periodic acid treatment.

RESULTS

Enzymes found in the subcutaneous fat bodies of the chick (see Table 1):

TABLE 1
HISTOCHEMICAL RESULTS IN EMBRYONIC CHICK ADIPOSE TISSUE

Alkaline Phosphatase			Acid Phosphatase		Esterase		
Age of Chick Embryo	Time of Incubation	Results	Time of Incubation	Results	Time of Incubation	Results	Glycogen
13 days	6 hrs.	+	20 min.	+	7.5 hrs.	+	
14 "			2 hrs.	+			
			45 min.	+	1 hr.	—	—
15 "	1 hr.	—	45 min.	+	1 hr. ?		
16 "	1 hr.	—	20 min.	+	7.5 hrs.	+	
	11 hrs.	+	1.5 hrs.	+	23.5 hrs.	+	
17 "	5 hrs.	—	15 min.	+	14 hrs.	—	—
	24 hrs.	+	30 min.	+			
18 "	1 hr.	—	40 min.	+	24 hrs.	+	—
19 "	15 min.	—	15 min.	+	1 hr.	—	—
	30 min.	—	30 min.	+			
	60 min.	—	60 min.	+			
20 "	2 hrs.	+	15 min.	+	1 hr.	+	—
21 "	1.5 hrs.	+			4 hrs.	—	
day old			15 min.	+			
chick	1 hr.	—	30 min.	+			
			60 min.	+			
2 day old					1.5 hrs.	+	
chick	12.5 hrs.	+	30 min.	+	12.5 hrs.	+	

(+) in the alkaline phosphatase column signifies staining of fat cells and other cell nuclei. In the acid phosphatase column it represents stain in the nucleus or cytoplasm of fat cells. For lipase it represents the presence of precipitate in the fat cells.
(—) indicates there was no stain or precipitate in the fat cells.
(?) indicates questionable results.

Alkaline phosphatase.—This enzyme was detected only in the wall and lumen of blood vessels of the fat bodies although with incubation of two hours and longer, a black precipitate was observed in the nuclei of fat cells, striated muscle fibers and fibroblasts. Such nuclear staining was obtained at 13, 16, 17, 20 and 21 days of incubation and in the 2-day old chick. The cytoplasm was usually stained gray and occasionally black.

Acid phosphatase.—This enzyme was demonstrated at each embryonic stage and in the newly-hatched chick. The nuclei and occasionally the cytoplasm of fat cells contained black granules of lead sulfide (Figs. 3, 4). In unfixed sections the cytoplasm was filled with these granules, and the nuclei could not be distinguished. In some sections

brown granules were observed also in the nuclei of erythrocytes, endothelial cells and fibroblasts. In one *control* section of the 19-day tissue there were nuclei stained with brown granules in one small area near the surface. This section had been incubated in subtrate medium with added fluoride. This staininig never occurred on any other control section, so it appeared to have been due to lead adsorption.

Esterase.—Esterase activity was detected at 13, 16, 18 and 20 days and in the 2-day old chick. At 14, 17, 19 and 21 days the sections were negative. Inconclusive results were obtained at 15 days. Enzyme activity was represented by clumps of lead sulfide in the form of granules and of needle-like crystals.

Glycogen.—None was detected at 14, 15, 17, 18, 19 and 20 days of incubation.

Enzymes found in the brown adipose tissue of the mouse (see Table 2):

TABLE 2

HISTOCHEMICAL RESULTS IN EMBRYONIC BROWN ADIPOSE TISSUE OF THE MOUSE

Alkaline Phosphatase			Acid Phosphatase		Esterae		
Age of Mouse Embryo	Time of Incubation	Results	Time of Incubation	Results	Time of Incubation	Results	Glycogen
16 days	30 min.	+	15 min.	—	2 hrs.	?	—
	60 min.	+	30 min.	light stain			
	1.3 hrs.	+					
17 days	15 min.	+	60 min.	light stain	1 hr.	—	—
	60 min.	+					
*18 days	30 min.	+	30 min.	?	1.5 hrs.	+	+
	40 min.	+					
	60 min.	+					
19 days	30 min.	—	27 min.	light stain	2.3 hrs.	?	—
*20 days	2.5 hrs.	+	30 min.	light stain	4 hrs.	?	+
*21 days	2.5 hrs.	+			4 hrs.	?	—
1.5 days young mouse	1 hr.	—	35 min.	light stain	3 hrs.	+	—
6.5 day young mouse	1 hr.	—	45 min.	—	8.5 hrs.	+	—
*Adult	30 min.	—	27 min.	light stain	9 hrs.	+	—
	60 min.	—					
	23 hrs.	+		+			

The (+) and (—) signs are used as in Table 1.

* The staining of the 18-, 20-, 21-day and adult stages for alkaline phosphatase is considered due to diffusion from nearby blood vessels. "Light stain" indicates a light brown staining of the nucleoli of the fat cells. The (+) sign in the glycogen column represents red stain or granules in the fat-cell cytoplasm.

(c) indicates questionable results.

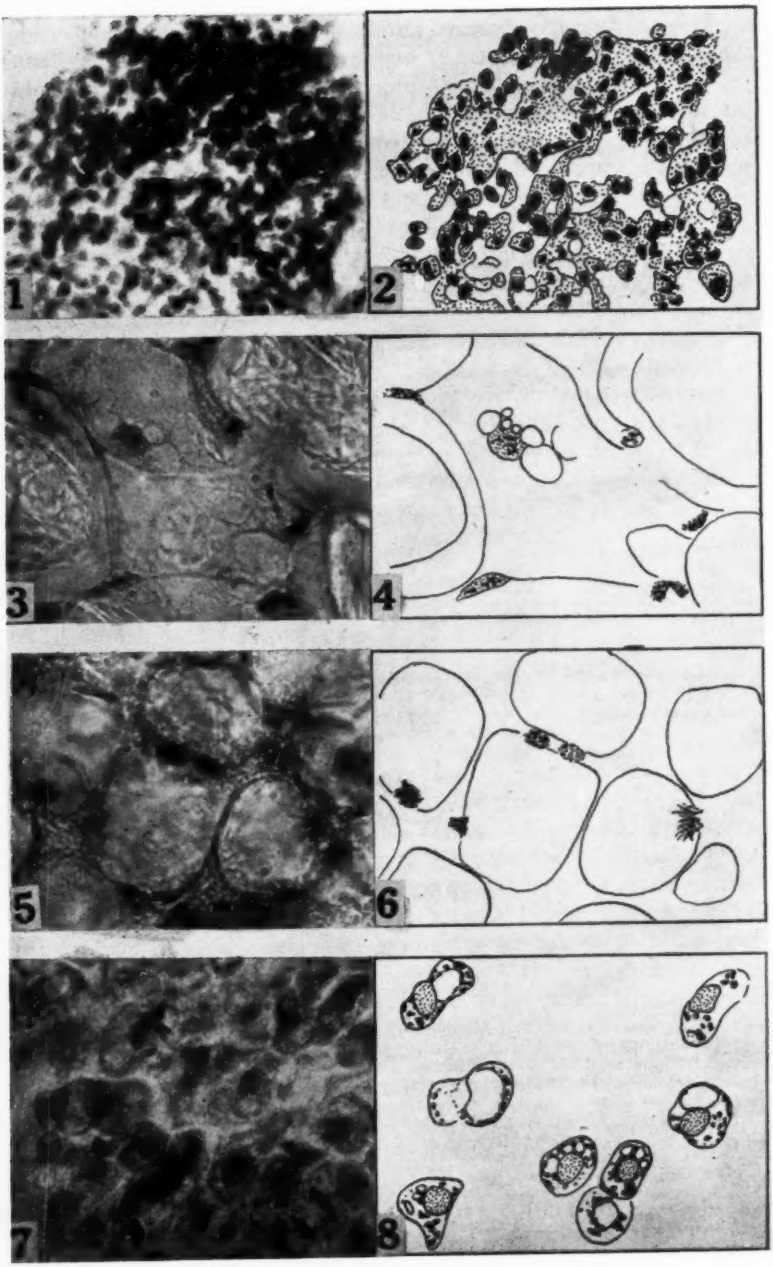
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PLATE I

Explanation of Figures

Figures 1, 3, 5 and 7 are unretouched photomicrographs, and Figures 2, 4, 6 and 8 are corresponding drawings of a part of the same section.

Figs. 1-2. Section of interscapular brown fat body of 16-day mouse embryo, fixed in acetone, incubated 30 minutes in glycerophosphate at pH 9. Alkaline phosphatase activity represented by black stain in nuclei and cytoplasm of fat cells. No fat vacuoles can be observed. No counterstain. ×430.

Figs. 3-4. Adipose tissue of 16-day chick embryo, fixed in acetone and incubated 20 minutes at pH 5. Acid phosphatase activity represented by black granules in nuclei of fat cells. Note multivacuolar fat cell in contrast to large monovacuolar cell. Eosin counterstain. ×970. Oil immersion.

Figs. 5-6. Cells of adult interscapular brown fat body, fixed in acetone and incubated 9 hours at pH 7. Esterase activity is represented by clumps of lead sulfide precipitate, seen readily in the drawing. Eosin counterstain. ×970. Oil immersion.

Figs. 7-8. Paraffin section, 7μ thick, of interscapular brown adipose tissue of 20-day mouse embryo stained for glycogen by the Periodic acid-Schiff reaction. Coarse granules which represent glycogen can be observed in the cytoplasm of the fat cells. Note the multivacuolar nature of fat cells in the lower part of the drawing. Hematoxylin counterstain. ×970. Oil immersion.

Alkaline phosphatase.—The enzyme was active at 16 and 17 days gestation, as indicated in the adipose tissue by a black stain, while muscle fibers and connective tissue were unstained (Figs. 1, 2). At 18 and 19 days the sections were a diffuse, brown color with a few black areas adjacent to blood vessels. At 20 and 21 days, the sections incubated 2.5 hours presented heavily stained blood vessels surrounded by fat cells with stained nuclei. Other areas were a pale brown color. Only blood vessels were stained black in the 6.5-day old mouse. Adult brown adipose tissue was stained only in the capillaries except after incubation of six hours or more when the nuclei assumed a black color.

Acid phosphatase.—No positive results were obtained except in the nuclei of fibroblasts and muscle fibers at 16 days and in the adult where nuclei of fat cells and endothelial cells were stained. At other stages, sections were stained a light brown color with a distinct but light stain on the nucleoli of fat cells. The controls were pale versions of these.

Esterase.—Positive esterase activity was obtained with sections of the 18-day stage, the 1.5- and 6.5-day old mice as well as in the adult. The precipitate of lead sulfide was distributed through the cytoplasm (Figs. 5, 6). Inconclusive results were obtained in 16, 17, 19, 20 and 21 day embryos.

Glycogen.—At 18 days the cytoplasm of scattered fat cells was stained a homogeneous, reddish-purple color. At 19 days only muscle fibers were positive. At 20 days most fat cells contained many large and small red granules in the cytoplasm (Figs. 7, 8). Both the brown and white adipose tissues of a mature female were negative.

Discussion

If an enzyme appears at a given embryonic stage and later vanishes or decreases greatly in activity before the end of embryonic life, it might be assumed that the enzyme participates in some developmental process. Alkaline phosphatase in the interscapular brown fat bodies of the mouse embryo at 16 and 17 days conforms with this pattern (Table 2). After incubation periods of 15 and 30 minutes, the nuclei and cytoplasm of brown adipose fat cells of 16 and 17 day embryos were darkly stained (Figs. 1, 2). These results appear to be valid since control sections of embryonic mouse kidney were comparably stained after being incubated 10 minutes. At 18 days the isolated areas of stained fat cells are probably the result of diffusion and

adsorption of calcium phosphate from the capillaries. At 19 days sections were negative, but at 20 and 21 days, incubation of 2.5 hours yielded more extensive but similarly stained fat cells around blood vessels, while peripheral areas were unstained. Therefore, these positive results appear to be diffusion and adsorption artifacts, and the positive stain in the adult tissue after 23 hours incubation is put in the same category.

Simultaneously with this early enzyme activity was a rapid proliferation, for numerous mitotic figures were present at 16 and 17 days gestation. There were a few at 18 days and rare ones at 19 days. This suggested that the enzyme might be taking part in cell division and proliferative processes as Moog (1944, 1946a, 1946b) has indicated earlier. Tsuboi (1952) presented evidence of a higher level of alkaline phosphatase in regenerating mouse liver than in the normal, similarly indicating that the enzyme may participate in growth and regenerative processes.

Esterase activity at 18 days and in the newborn and adult mouse did not suggest a relationship with embryonic processes. However, fat vacuoles appear first at 18 days simultaneously with the enzyme. Possibly the enzyme is present from 18 days to parturition since lipid vacuoles are present throughout this period. Von Richterich (1952) is of the opinion that both lipase and nonspecific esterases may be revealed by the Gomori technique and that false results may be obtained by the diffusion of these enzymes or their products or by nonspecific reactions of other substances. In view of these several possibilities no final concluion was reached concerning the role of esterase in developing fat bodies.

The appearance of glycogen in the interscapular fat bodies of the mouse embryo just prior to term, may be related to the time of birth rather than to lipid synthesis; i.e. it may be an energy reserve utilized for the first activities of the newborn. The disappearance of glycogen at 19 days may be misleading because the method used to determine the age of mouse embryos is not precise. Our 19-day tissue may have been 19 days $\pm$ 15 hours since the males and females were paired for about 15 hours, and the age of the embryo was counted from the night the pair was placed together.

The slight indication of acid phosphatase in brown fat bodies is not positive enough to warrant comment. However, in the chick fat bodies the presence of the enzyme was plainly indicated in both the

cytoplasm and the nucleus. In contrast to this finding, several workers have found by chemical analysis that the phosphatases are confined to the cytoplasm in some tissues (Novikoff *et al.*, 1950; Palade, 1951; Stern *et al.*, 1952). Others have demonstrated that nuclear staining is often an artifact in the histochemical detection of acid and alkaline phosphatase (Martin and Jacoby, 1949; Newman *et al.*, 1950; Macdonald, 1950; Novikoff, 1951; Gomori, 1951). However, there are other tissues in which the phosphatases have been found in the nucleus, e.g. horse liver (Stern *et al.*, 1952), in cells of the cerebral cortex (Richter and Hullin, 1951), and in chicken erythrocytes (Dounce and Seibel, 1943; Rabinovitch *et al.*, 1948).

That acid phosphatase may participate in carbohydrate metabolism in chick fat bodies is suggested by the fact that the phosphatases hydrolyze some of the substrates known to take part in glycolysis. Meyerhof and Green (1949a, 1949b) demonstrated that both enzymes will synthesize as well as hydrolyze glucose-1-phosphate, fructose-1-phosphate, glucose-6-phosphate and alpha glycerophosphate; also the alkaline enzyme will take part in transphosphorylation. That glycolysis probably occurs in adipose tissue was shown by Mirsky (1942), and by Creasey and Gray (1951) who detected glycolytic enzymes in white and brown adipose tissue of mammals. The same enzymes might be anticipated in avian adipose tissue. In addition Cater and Lawrie (1951) detected glycogen and acid phosphatase in the chick preen gland.

Summary

1. Alkaline phosphatase was detected in the interscapular fat bodies of the mouse embryo at 16 and 17 days gestation. Little activity was detected in the succeeding stages up to term, in the newborn mouse or the adult. Slight activity of acid phosphatase was indicated in the various embryonic stages to term and moderate activity in the adult. Esterase activity was detected at 18 days gestation in the newborn, and in the adult mouse. Glycogen was found in the interscapular gland of the embryo just before birth.

2. In the subcutaneous fat bodies of the chick, except for positive results in small blood vessels, no evidence for alkaline phosphatase was obtained at any embryonic stage or in the newly-hatched chick. Acid phosphatase was detected at all embryonic stages, in the nuclei of fat cells and in the nuclei of endothelial cells and erythrocytes. In

unfixed adipose tissue, acid phosphatase was found in the cytoplasm. Esterase activity was detected at 13, 16, 18 and 20 days of incubation as well as in the newly-hatched chick. Glycogen was not detected at any stage.

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BOOK REVIEW

Morphogenesis, An Essay on Development, by John Tyler Bonner. Princeton University Press, 1952. 296+vi pp. 90 figures.

REVIEWED BY EDMUND W. SINNOTT

This stimulating little book deals with what is generally agreed to be the central problem of biology—the question of growth, development and organic form. Darwin said that morphology was the soul of natural history, but I think he would admit today that this distinction should go to morphogenesis, the subject of Dr. Bonner's volume. Form is the fundamental problem, but form as process, not merely as static product.

The author breaks his subject down into the three phenomena of growth, morphogenetic movement and differentiation. The last he defines, too narrowly I think, as "an increase in the detectable differences in chemical composition of parts of an organism during development." Spatial as well as chemical differences are surely manifestations of differentiation. Morphogenetic movement itself may perhaps be regarded as one aspect or source of the more basic phenomenon of differentiation.

The author presents with conciseness and clarity a considerable series of examples of organic patterns and their development. It is obviously impossible in such small compass to consider all the aspects of his vast subject but his discussion of these examples serves to pose most of the problems of morphogenesis. Since Dr. Bonner is a botanist he draws much more of his material from the plant kingdom than is usual in such treatments.

The many physical and chemical agencies or analogies which have been suggested as "explanations" of morphogenetic phenomena are discussed—surface tension, diffusion, molecular configuration (with special emphasis on the paracrystalline state), size, mechanical factors, hormones, genes, polarities, gradients, bioelectric fields, organizers and the rest. Unlike some over-enthusiastic protagonists he does not try to account for everything by any one agency but maintains a critical and impartial attitude toward all of them, showing how each is useful in accounting for certain facts but pointing out its weakness in explaining others. Indeed, he shows that quite different fac-

tors seem to be involved in producing the same phenomenon, as in the case of polarity. He also emphasizes the often disregarded fact that in such an agency as auxin, though it doubtless is importantly concerned in many processes, the cause of its own spatial and temporal distribution—vital in any final "explanation"—is unknown. "It is one thing," he remarks, "to say that water runs down hill because the hill is inclined, and it is another to explain why the hill is inclined."

Dr. Bonner comes back repeatedly and with refreshing candor to a confession that the fundamental problem of development—organization—is still quite unsolved. He does not like the word "organization" itself for to him it is only an expression of ignorance; but since we are admittedly ignorant here, it seems to this reviewer the best term available. "Development," "regulation," "differentiation," "wholeness,"—these are all concerned with one facet or another of the more general phenomenon of biological organization. That the basic problem of morphogenesis *is* unsolved continues often to be treated with an embarrassed silence. As Dr. Bonner has recently said elsewhere, "We understand no better than Driesch did the explanation of a harmonious equipotential system, and yet half a century of embryology has elapsed."

This difficulty does not drive him to defeatism or vitalistic theory. He is confident that in due time an explanation in terms of physics and chemistry will be forthcoming. He is intrigued, as so many others have been, with the analogy between organisms and crystals and is inclined to think that when we have learned much more about the micro-structure of protoplasm we may find in it the clue to our enigma. This certainly is a hopeful lead and follows the sound confidence of science that analysis, carried further and further, will continue to be fruitful. But always in the end we are left with smaller and smaller pieces, and miss the secret of the whole. The theory of the gene was a tremendous advance in an understanding of heredity but we now know that it was only the beginning. Synthesis rather than analysis is what is needed now. Even if we could carry a knowledge of organic micro-structure down to the atomic level, we might still be frustrated, for let us not forget that the atom itself is a complex system the organization of which is far from understood.

This book is delightfully written and is packed with information and ideas. In covering so much ground it necessarily suffers from a certain lack of logical order, but it will be greatly useful in acquainting both scientists and laymen with some of the most difficult, fascinating and vitally important problems in biology.